THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

European Reference Network for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

Present and future of gene therapy in Neuromuscular Diseases

Satellite Scientific Symposium endorsed by ERN EURO-NMD

February, 22nd 2024

Working Group Neuropathies

Working Group Gene Therapies

Gene Therapies for peripheral neuropathies
Present and future

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NEUROMUSCULAR DISORDERS CENTER

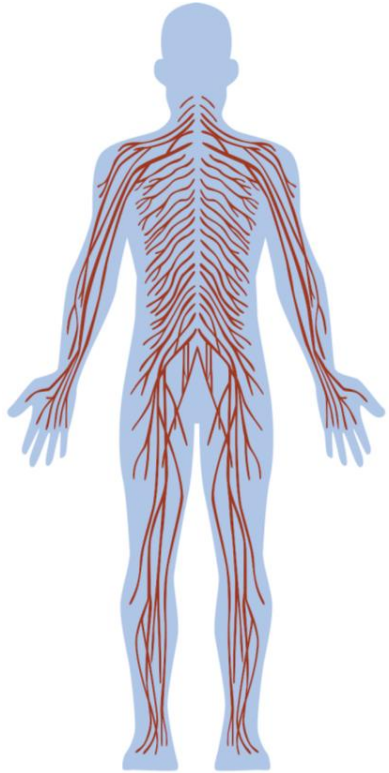
& Department of Neuroscience

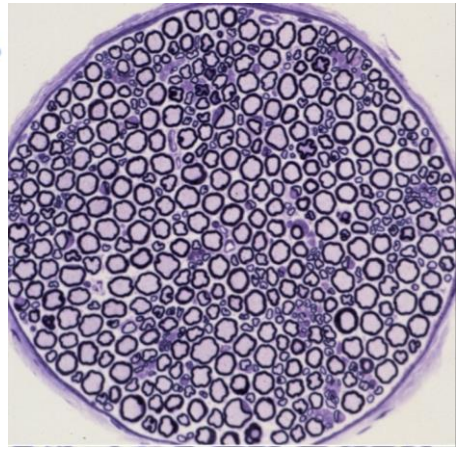
THE CYPRUS INSTITUTE OF NEUROLOGY AND GENETICS

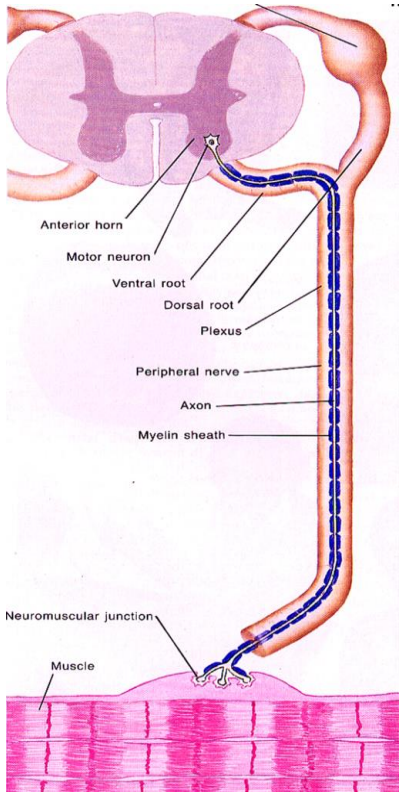
Nicosia, CYPRUS

CMTR Charcot-Marie-Tooth and Related Neuropathies

Gene therapy developments in peripheral neuropathies





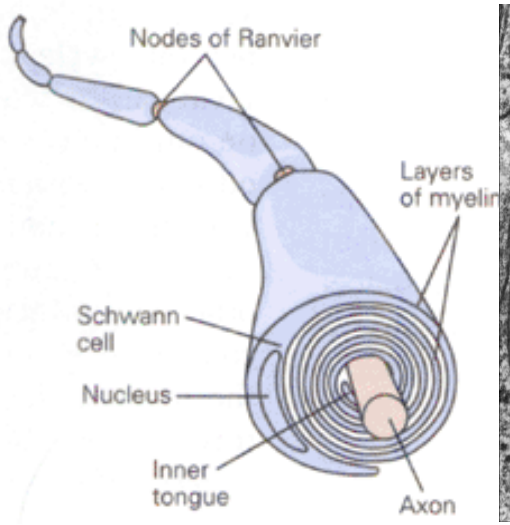


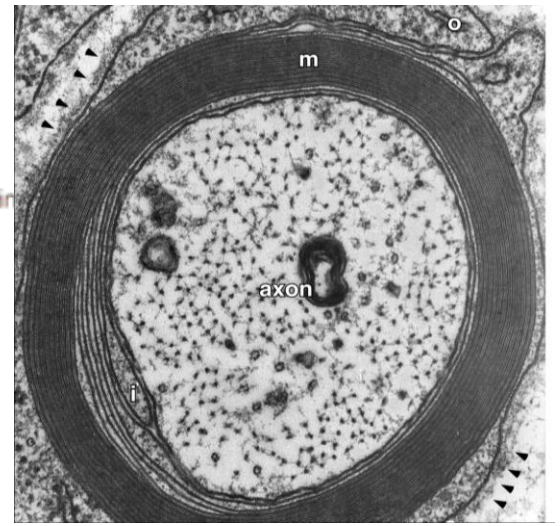
Syndromic neuropathies

- **Hereditary TTR variant amyloidosis (hATTRv)**
- **Giant axonal Neuropathy (GAN)**

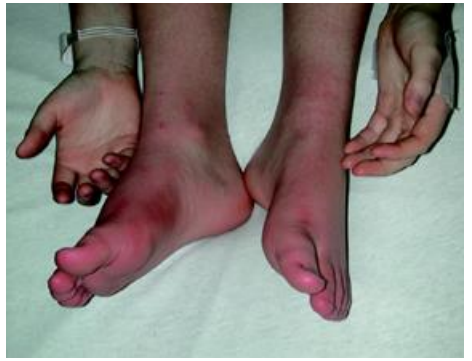
Non-syndromic neuropathies

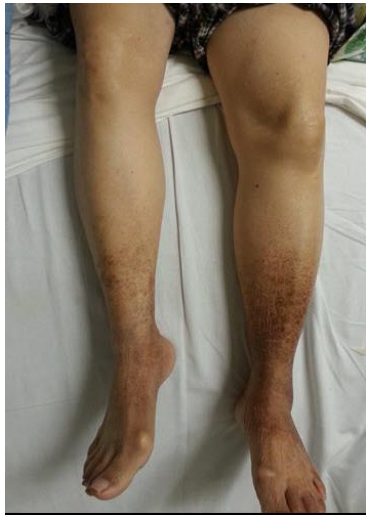
- **Charcot-Marie-Tooth (CMT) diseases**
  - Demyelinating CMT Types 1 and 4
  - Axonal CMT Type 2
  - Intermediate CMT



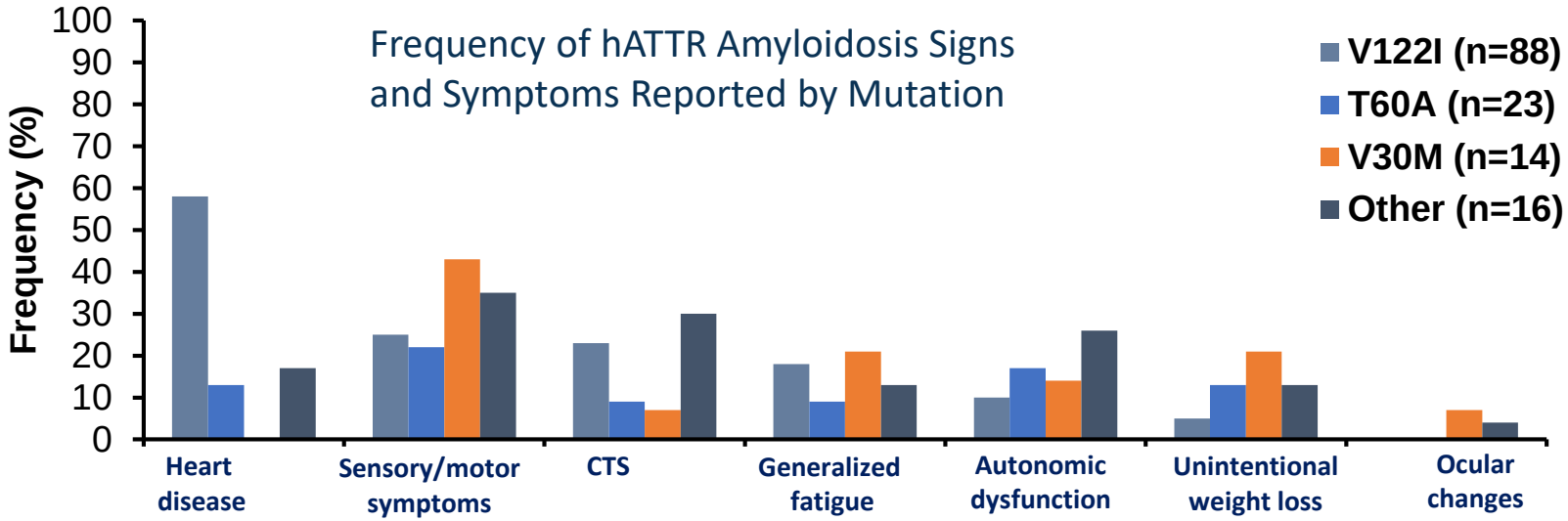


hATTRv Amyloidotic polyneuropathy

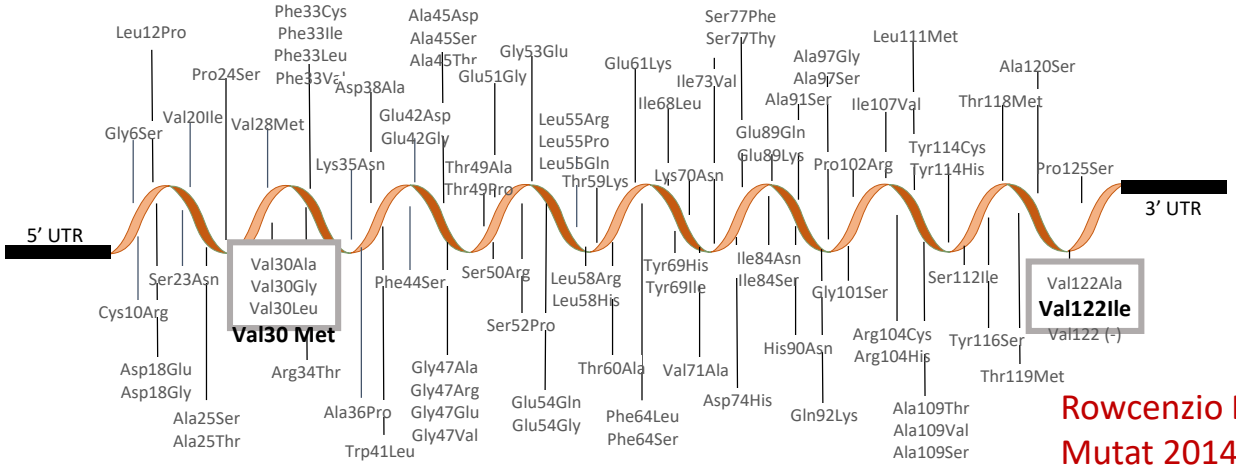




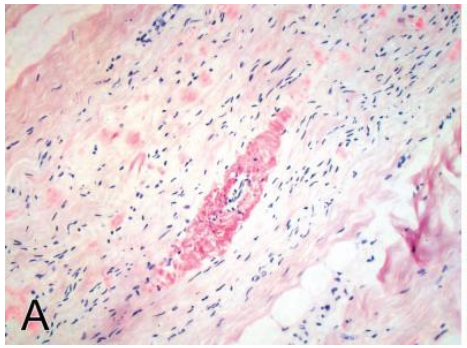
- hATTR Amyloidosis manifests with a variety of symptoms

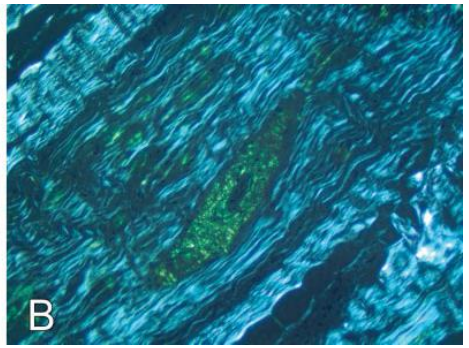


Data from Alnylam (http://www.alnylam.com/patients/alnylam-act/) Mora et al. *Neurology* 2018;90(15 Suppl.):P1.448



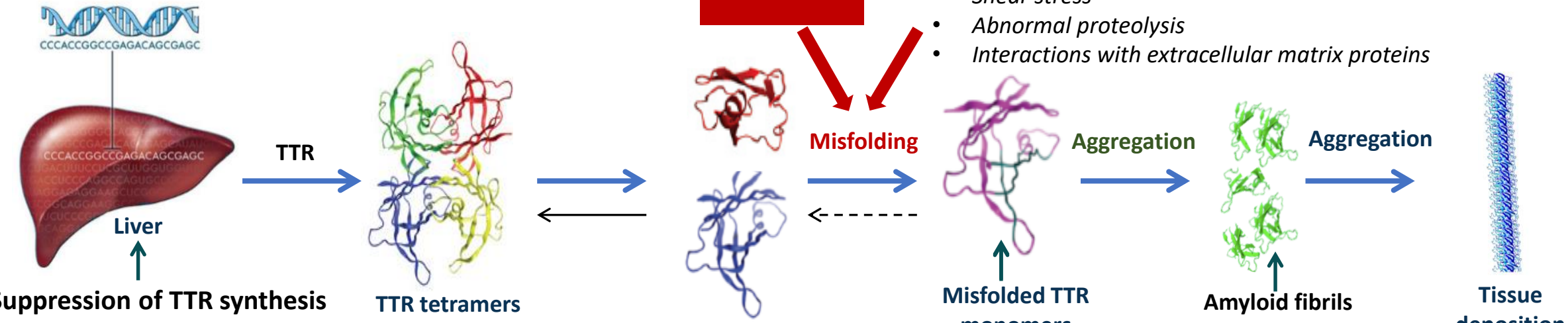
Rowcenzio DM, et al. Hum Mutat 2014;35:E2403-12





hATTR: molecular pathogenesis and therapeutic approaches

Misfolded mutant or wild-type TTR protein accumulates as amyloid deposits in nerves, heart, GI tract, and other tissues



Suppression of TTR synthesis

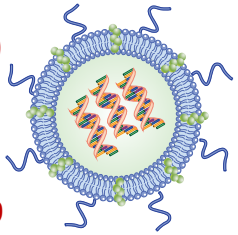
RNAi therapeutics

- **ONPATTRO™ (patisiran)**
- **AMUTTRA (vutrisiran)**

Anti-sense oligonucleotides

- **TEGSEDI™ (inotersen)**

Gene deletion: CRISPR-Cas9



knock down both mutant and WT TTR production

Orthotopic liver transplantation (OLT)

TTR stabilization

- **Tafamidis**
- **Acoramidis (AG10)**
- Diflunisal
- SOM0226

Fibril degradation and reabsorption (investigational)

- Doxycycline + TUDCA
- Anti-serum-amyloid protein (SAP) antibody
- Anti-TTR monoclonal Ab
- Epigallocatechin-3-gallate (EGCG)

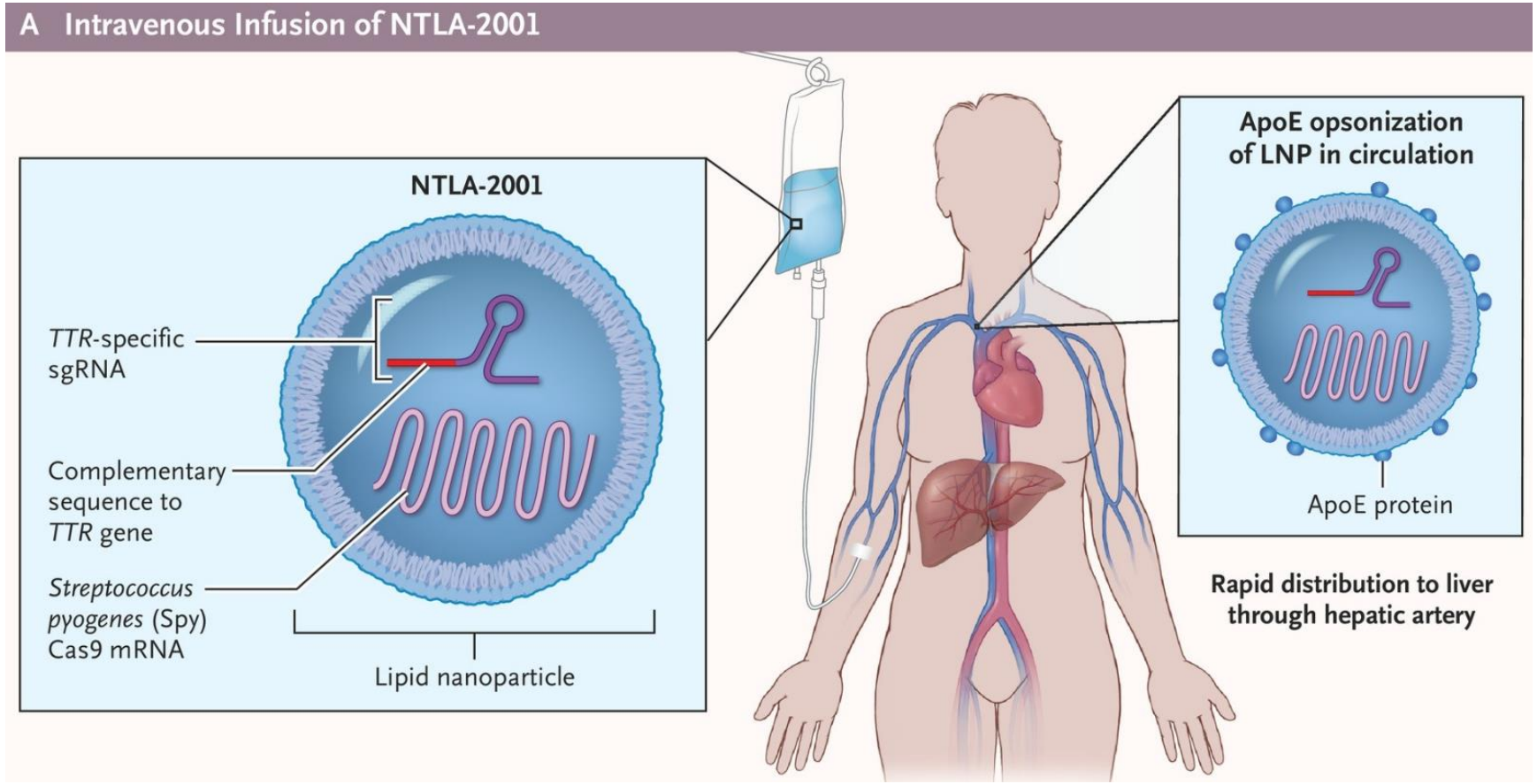
Conceição et al. *J Peripher Nerv Syst* 2016;21:5-9; 6. Swiecicki et al. *Amyloid* 2015;22:123-31; 1. Adams et al. N Engl J Med 2018;379:11-21; 2. Benson et al. N Engl J Med 2018; 379:22-31; 3. Ando et al. Orphanet J Rare Dis 2013;8:3; 4. Said et al. Nat Rev Drug Dis 2012;11:185–186; 5. Berk et al. JAMA 2013;310:2658–2667; Richards et al. N Engl J Med 2015;373:1106–1114

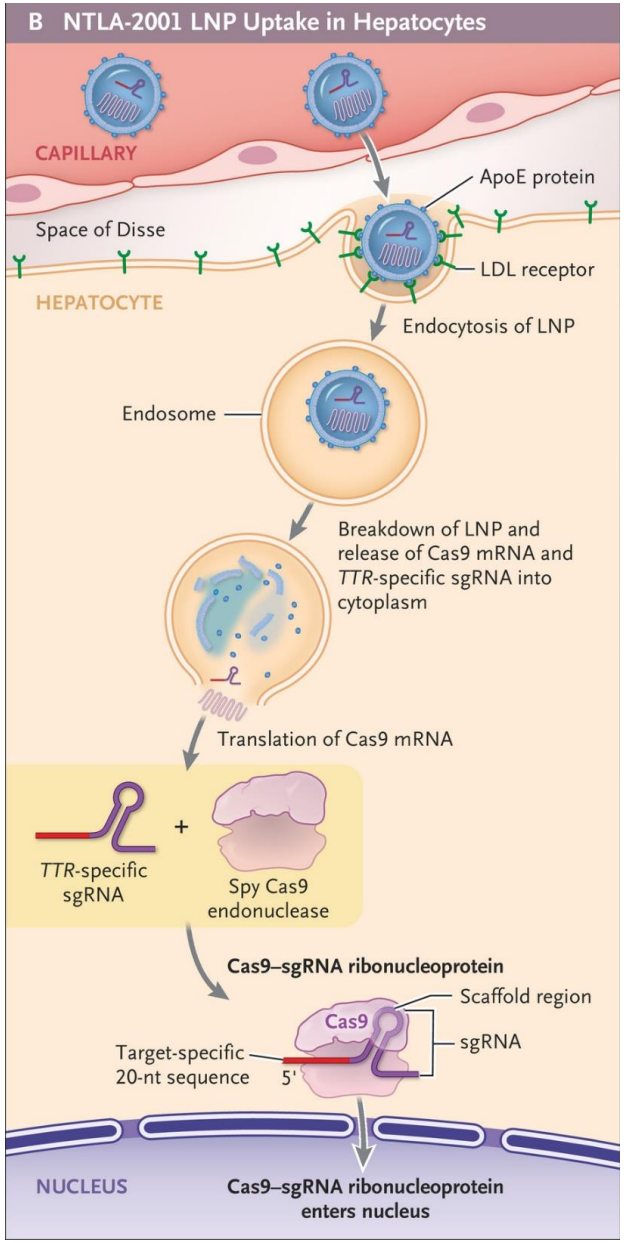
RESEARCH SUMMARY

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

Gillmore JD et al. DOI: 10.1056/NEJMoa2107454

NTLA-2001: lipid nanoparticle (LNP) delivery system with liver tropism, carrying a single guide RNA (sgRNA) that targets human *TTR* and a human-codon–optimized mRNA sequence of *Streptococcus pyogenes* Cas9 protein





Gillmore et al. NEJM 2021

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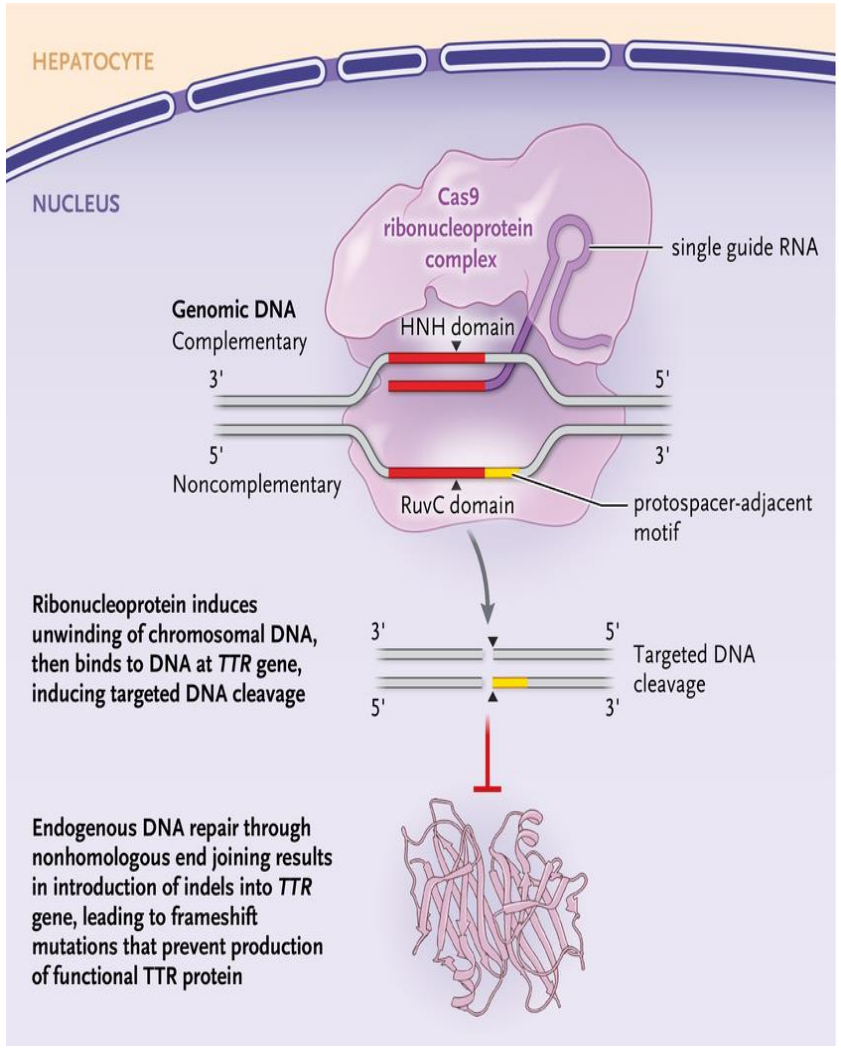
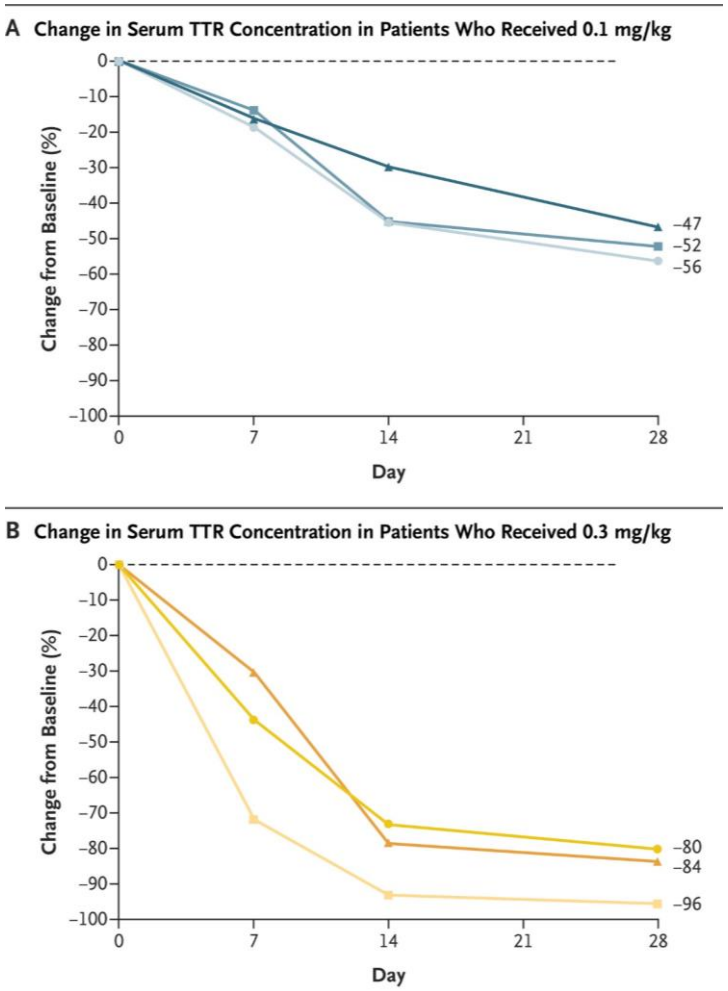


Gillmore et al. NEJM 2021



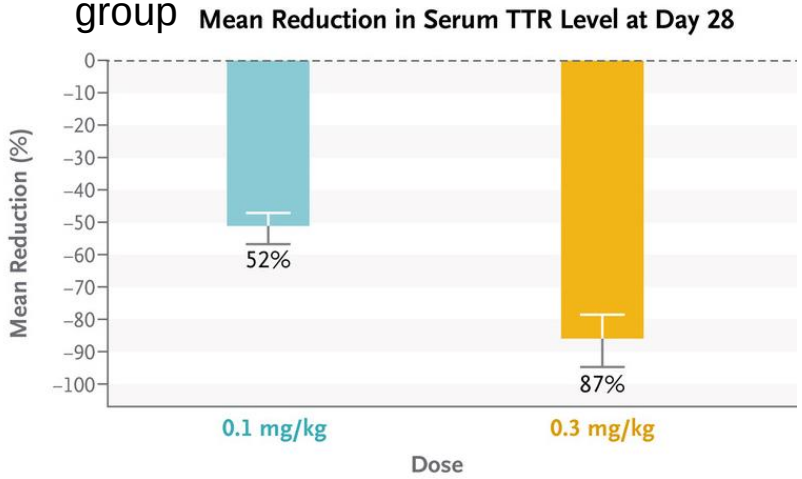
Phase I clinical study:

6 patients with hATTRv polyneuropathy

- 2 initial dose groups (n=3 of 0.1 mg/Kg and 0.3 mg/Kg)
- Ages: 53.5 years (46-64), 4 male, 2 female
- Polyneuropathy score 1 in all

Mean reduction from baseline in serum TTR protein concentration:

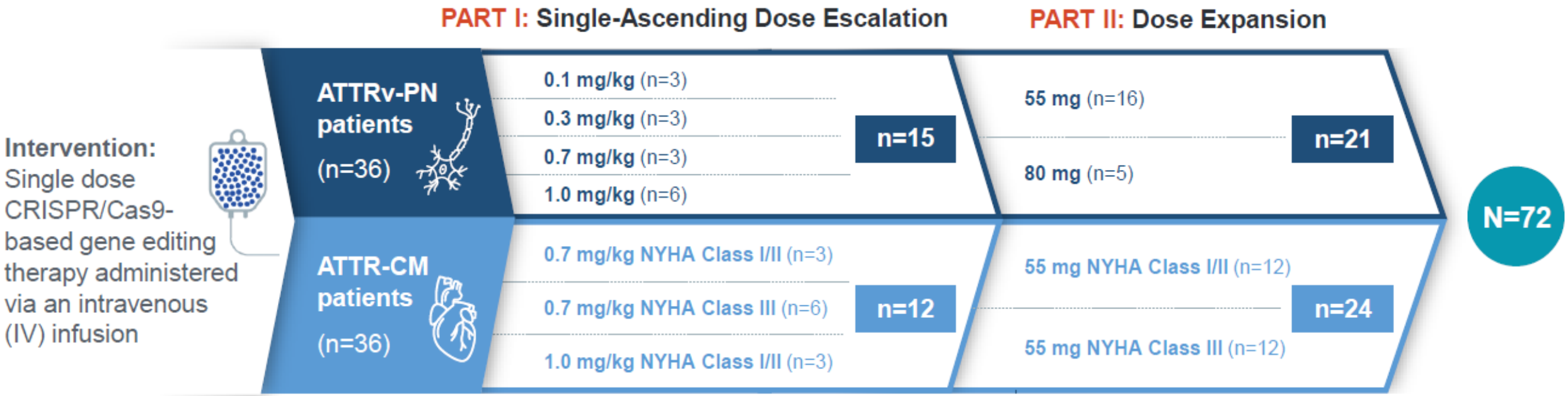
- 52% (47-56) in the 0.1 mg/Kg dose group
- 87% (80-96) in the 0.3 mg/Kg dose group



Two-part, open-label, multicenter study in adults with hereditary ATTR amyloidosis with polyneuropathy (ATTRv-PN) or ATTR amyloidosis with cardiomyopathy (ATTR-CM)

Intellia Therapeutics

- **Ongoing Phase 1, two-Part: Open-label, Single Ascending Dose (Part 1)** and **Open-label, Single Dose Expansion (Part 2)** Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NTLA-2001 in Patients with ATTRv-PN and Patients with Cardiomyopathy (ATTR-CM)
- **72 patients already** treated with **4 dose-escalation cohorts**
- **expected completion in 2025**



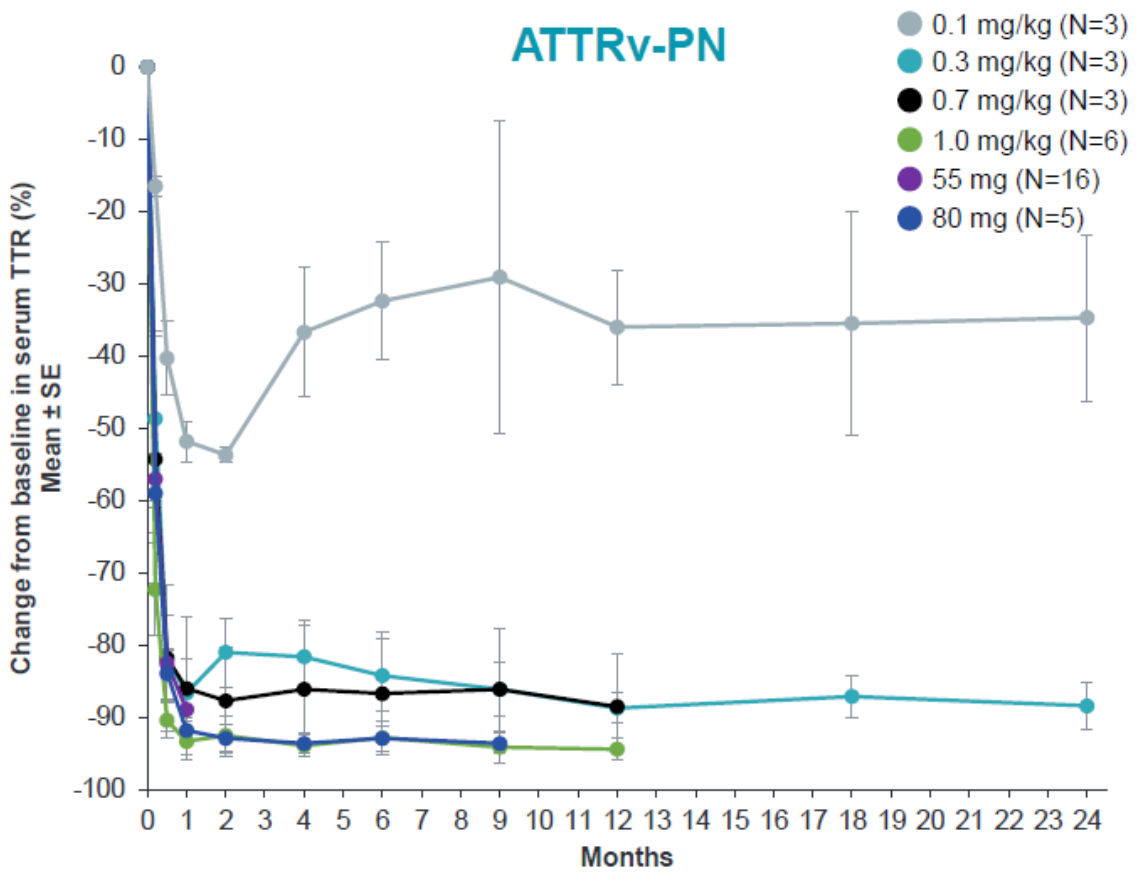
Presented by *Gillmore et al. Nov 2023*

CRISPR-Cas9 in vivo gene editing for ATTRv

Patient demographics and characteristics (PN arm)

Characteristic		PN Patients (N=36)
Age, years	Median (min, max)	61 (19, 75)
Sex, n (%)	Male	26 (72)
Weight, kg	Median (min, max)	77 (55, 117)
***TTR* genotype, n (%)**	p.V50M	11 (31)
	p.V142I	1 (3)
	p.T80A	7 (19)
	p.S97Y	7 (19)
	p.E62D	4 (11)
	Other	6 (17)
	WT	0
NYHA Class, n (%)	No diagnosis of heart failure	12 (33)
	I	19 (53)
	II	5 (14)
	III	0
NT-proBNP, ng/L	Median (min, max)	127 (<50, 1878)

- NTLA-2001 led to **sustained reduction of TTR levels** in all patients and dose groups – but weaker reduction in the 0.1 mg/Kg group (data from 33 patients)



Presented by *Gillmore et al. Nov 2023*

CRISPR-Cas9 in vivo gene editing for ATTRv: Safety and Side-Effect Profile

❑ From the published 2021 study

- Adverse events occurred during or after treatment in 3/ 6 patients, mild (grade 1) (Nausea, Headache, infusion related reaction)
- Increased D-Dimer levels observed 4-24 hours after infusion in 5 /6 patients, returned to normal by day 7
- Live function (ALS and AST levels) remained within normal limits

Gillmore et al. NEJM 2021

Potential for off-target gene editing with CRISPR-Cas systems?

- Therapeutic concentrations of **NTLA-2001 showed no off-target mutagenesis mechanisms in primary human hepatocytes**

➢ ***Need to undergo long-term safety monitoring!***

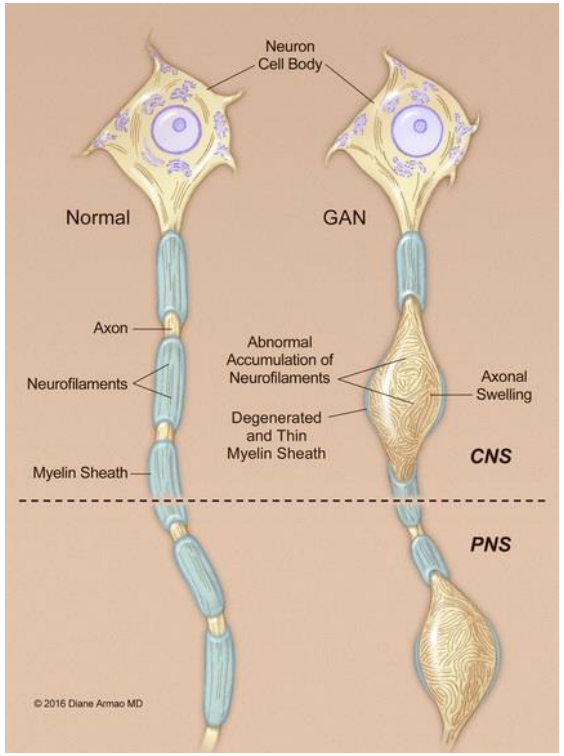
❑ Preliminary safety data from the ongoing multicenter study

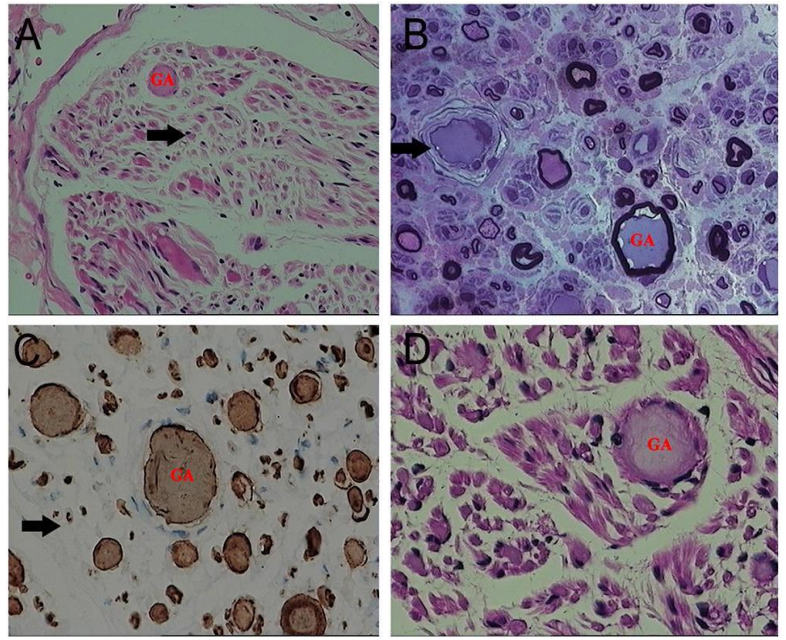
AE, Preferred Term, n (%)	Any Grade	Grade 1	Grade 2	Grade ≥3
Infusion-related reaction	25 (38)	10 (15)	14 (22)	1 (2)
Headache	12 (18)	12 (18)		
Diarrhea	11 (17)	10 (15)	1 (2)	
Back pain	7 (11)	7 (11)		
COVID-19 infection	6 (9)	5 (8)	1 (2)	
Cardiac failure	6 (9)	2 (3)	2 (3)	2 (3)
Upper respiratory tract infection	6 (9)	6 (9)		
AST increased	5 (8)	3 (5)	1 (2)	1 (2)
Dizziness	5 (8)	5 (8)		
Fatigue	5 (8)	5 (8)		
Muscle spasms	5 (8)	4 (6)	1 (2)	
Vision blurred	5 (8)	5 (8)		
Atrial flutter	4 (6)		1 (2)	3 (5)
Constipation	4 (6)	2 (3)	2 (3)	
Rash	4 (6)	4 (6)		

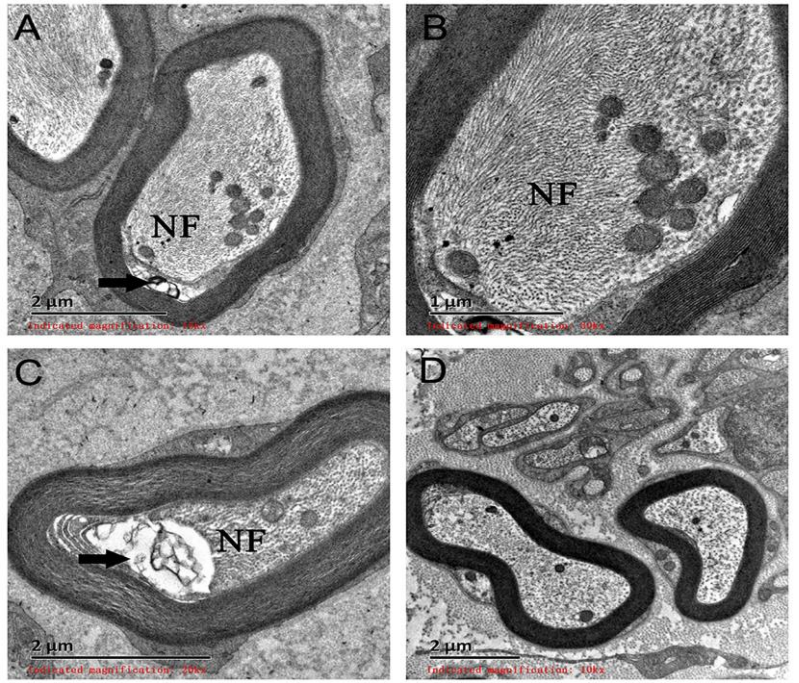
Presented by *Gillmore et al. Nov 2023*

Giant axonal neuropathy (GAN)

- Ultra-rare, autosomal recessive, progressive neurodegenerative disease with early childhood onset
- **Sensorimotor neuropathy** commonly progresses to affect both the PNS and CNS
- **Biallelic mutations in the *GAN* gene** located on 16q23.2, leading to loss of functional **gigaxonin**
- **Gigaxonin:** substrate-specific ubiquitin ligase adapter protein → regulates intermediate filament turnover







Loss of gigaxonin function

- **progressive accumulation of native intermediate filaments** in endothelial cells, skin fibroblasts, muscle fibres, Schwann cells, astrocytes and neurons
- **'Giant' axons or axonal swellings, within peripheral nerves, and in the CNS**: cerebral and cerebellar white matter, middle cerebellar peduncles, brainstem tegmentum, corticospinal tracts and posterior column (Asbury AK et al., 1972)

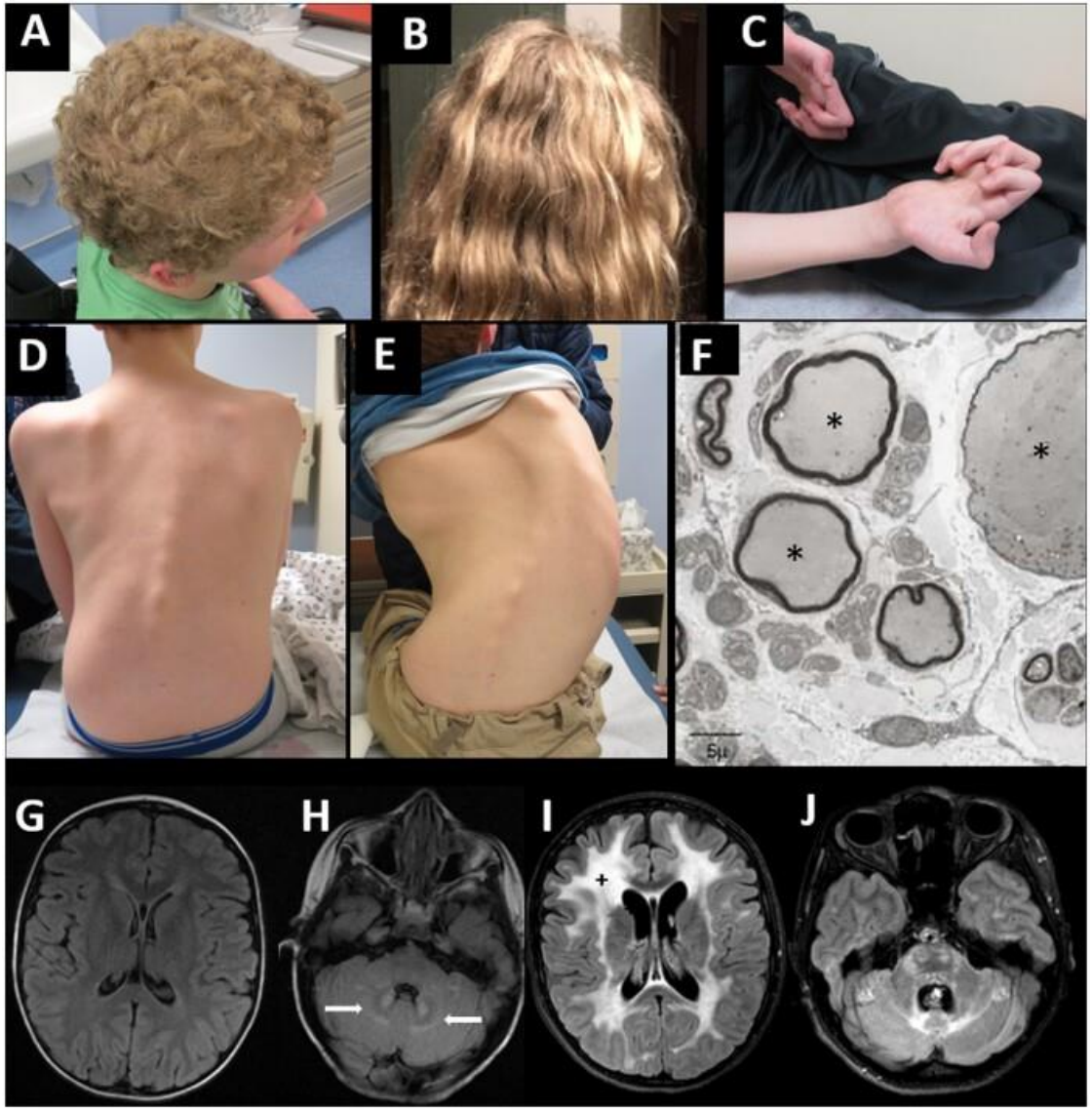
Zu et al., 2020

GAN clinical phenotype

Bharucha-Goebel et al Brain 2021: largest GAN cohort ***of 45 GAN patients*** *(ages 3-21)*

- **Mean** age at symptom **onset: 2.9 years**
- **PNS: Distal muscle weakness and sensory loss**
- Progressive **gait ataxia** in the ambulant individuals
- Age at **loss of unassisted ambulation: 8.3 years**
- **CNS:** Visual loss (22/45; 49%), dysarthria (19/45; 42%), urinary hesitancy or incontinence 13/45; 28%), precocious puberty (11/45; 24%), illness associated hypothermia (5/45; 11%) and sleep apnoea (14/45; 31%)
- **Seizures, vertigo, nystagmus, impaired cognitive development**
- Characteristic **dull, curly or tightly curled (frizzy) hair**
- **Gastrointestinal:** dysphagia (14/45; 31%), constipation (18/45; 45%); lactose intolerance (18/45; 40%)

Bharucha-Goebel et al Brain 2021



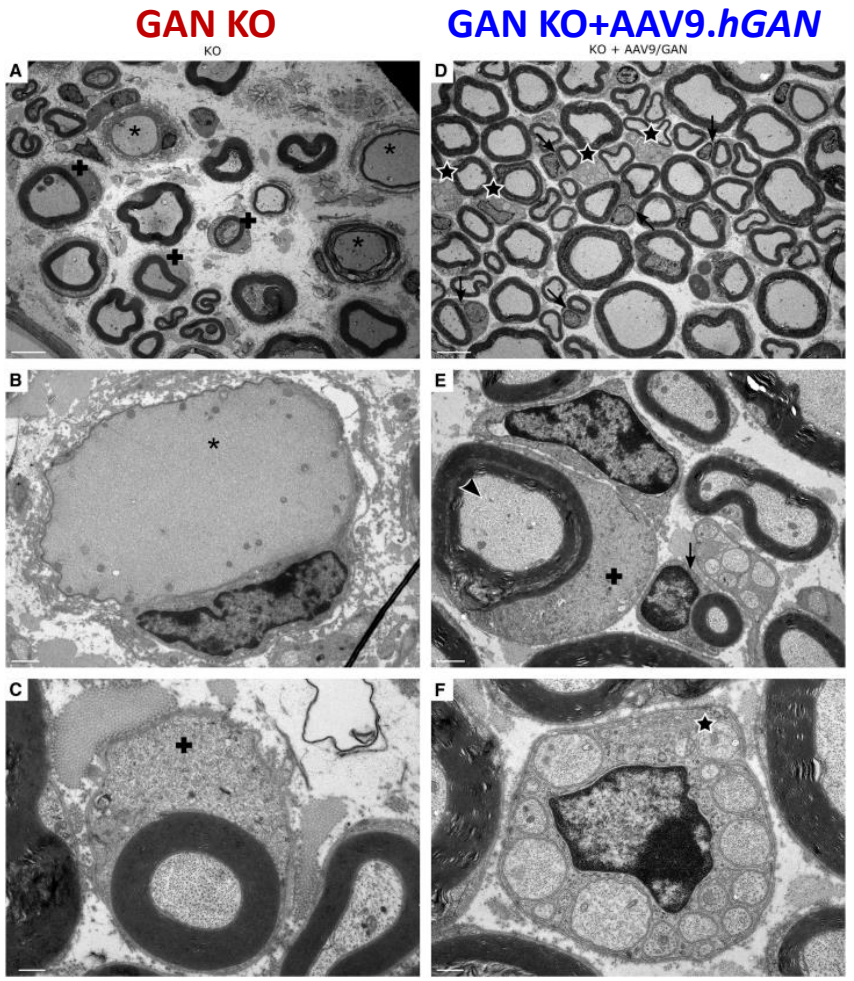
- Increased T2 signal abnormalities within cerebellar white matter surrounding the dentate nucleus of the cerebellum
- Cortical and spinal cord atrophy in more advanced disease severity

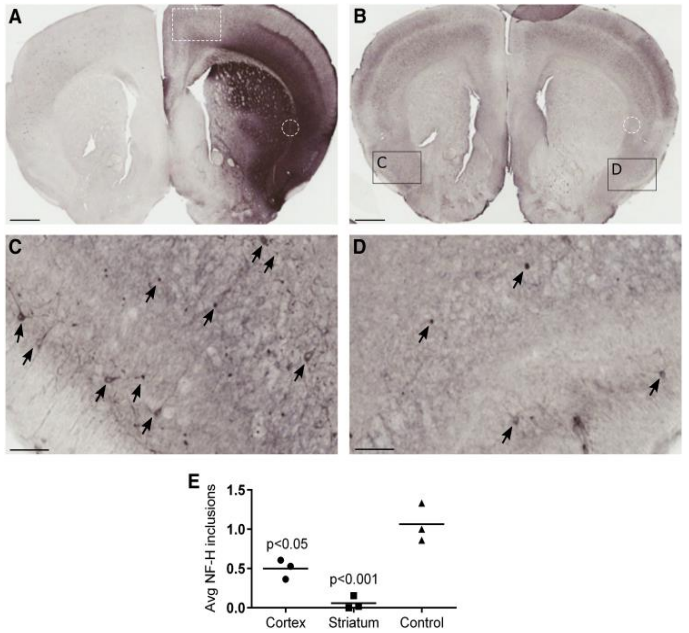
Development of GAN gene therapy

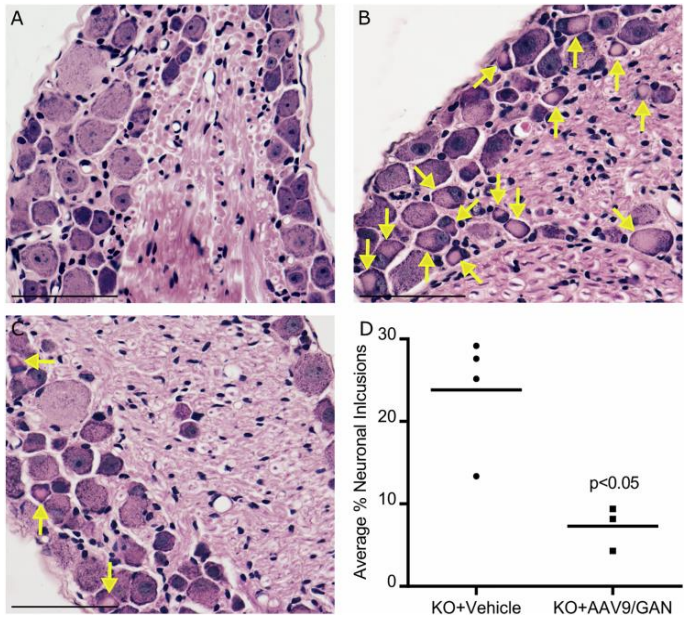
AAV/JeT-GAN gene transfer cassette

- AAV/JeT-GAN restored normal configuration of IFs in patient fibroblasts and in GAN KO mice
- IT delivery in aged GAN KO mice → preserved sciatic nerve ultrastructure
- → reduced neuronal IF accumulations
- → improved motor function
- Sustained wild-type gigaxonin expression in PNS and CNS for at least 1 year

Bailey et al., 2018







Clinical translation of GAN gene therapy

➢ **scAAV9-JeT-GAN intrathecal gene therapy was well-tolerated and seemed to slow disease progression in GAN patients: Data from Phase 1/2, first-in-human, single-site trial (NCT02362438)**

- **14 participants** with GAN dosed **IT** with either **$3.5x10^{13}$, $1.2x10^{14}$, $1.8x10^{14}$, or $3.5x10^{14}$ vg**
- Corticosteroid **immunomodulation**, plus T-cell targeted immune modulation in biallelic null variants patients (n = 4)
- **Primary endpoints: safety and change in motor function measure (MFM-32) score** at 1 year compared to baseline

Safety:

- **TEAEs:** abnormal VC, URTI, UTI, acidosis, hyperglycemia, leukocytosis, thrombocytosis, CSF pleocytosis (clinically silent and self-limited), headache, intracranial hypertension
- **1 serious AE:** fever and vomiting requiring i.v. fluids likely related to treatment
- **2 deaths due to pulmonary complications: deemed related to GAN and not the gene therapy**
- **Anti-AAV9 antibodies:** peaked at 1:2,560 to 1:327,680 titers, 12 participants with increased T-cell interferon (IFN)-γ response (lower in participants receiving T-cell immune suppression)
- No dose-dependent side effects

Efficacy (1 year post treatment):

- **3 highest dose groups combined had a significantly slowed annual decline in MFM-32 score** (*P* = .002)
- **Nerve biopsies (n=11)** showed the **presence of gene therapy** in regenerative nerve fibers
- **Sensory nerve action potentials (SNAPs) persisted and/or re-emerged** compared to a decline in natural history
- **Increased regenerative clusters** in superficial sensory nerves

Diana Bharucha-Goebel, MD et al., 2023 MDA Clinical & Scientific Conference, March 2023

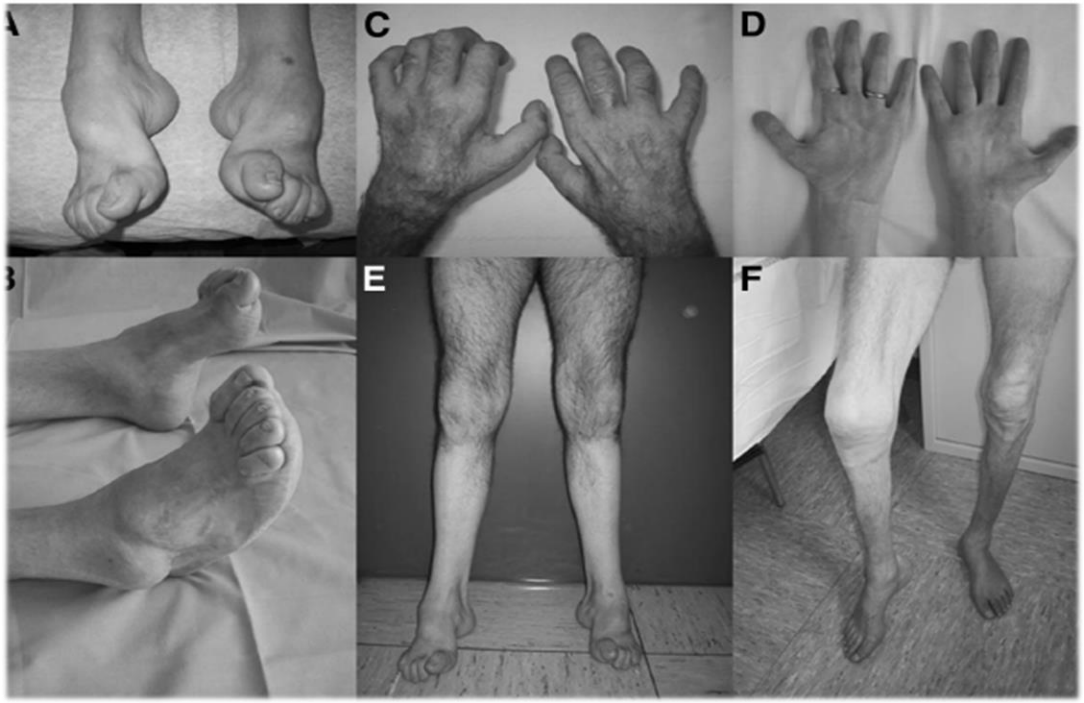
"Taysha Gene Therapies Halts Giant Axonal Neuropathy Drug Development on FDA Feedback"

! Lessons learned

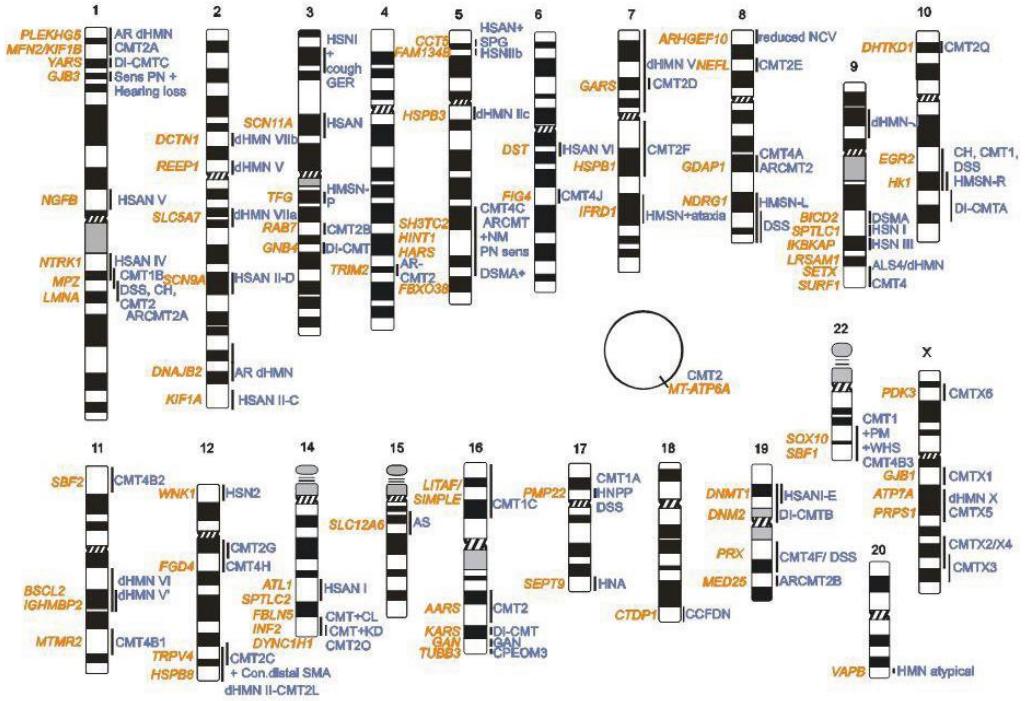
- **Taysha Gene Therapies discontinued in Sep 2023 development of TSHA-120, its gene therapy candidate for GAN, following feedback from the FDA on the company's intended registrational path.**
- "Due to challenges related to the feasibility of the study designs," (Taysha Chairman and CEO Sean Nolan)
- **Taysha submitted in 2022 available evidence from a Phase I/II trial** of TSHA-120 and reviewed the data with the FDA
- **FDA provided feedback:** company needs to address **patient heterogeneity in terms of disease progression in GAN** and **concerns about using MFM32 as an endpoint**
- **Taysha subsequently submitted another analysis of data from a natural history and interventional trial comparing functional and biological measurements against a disease progression model**
- **FDA continued to recommend a randomized, double-blind, placebo-controlled trial to demonstrate efficacy** and suggested **a potential path for a single-arm trial with an external control group** and recommended **longer-term follow-up**
- **However, Taysha has determined that discontinuing development of the gene therapy will reduce operating expenses →** now focuses on its gene therapy trial for Rett syndrome.
- Astellas Gene Therapies, which last year invested $50 million into Taysha, has elected not to exercise its option to obtain an exclusive license to TSHA-120

- ➢ **Careful clinical trial design!**
- ➢ **Well-designed Natural History Studies and carefully selected patients !**
- ➢ **Comprehensive, sensitive, regulatory acceptable outcome measures!**
- ➢ **Challenging financial environment, prioritize resources!**

Charcot-Marie-Tooth (CMT) neuropathies



Pareyson et al., 2006



Timmerman et al., 2014

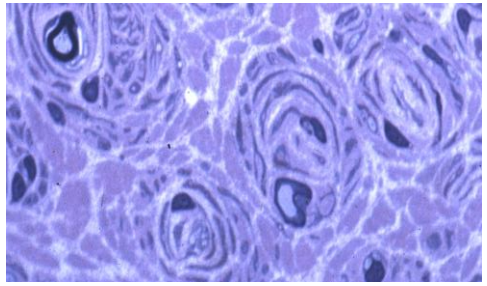
- **Prevalence 1:2500** worldwide
- **Onset** in childhood, latest in adolescence, very severe types may present in infancy (***range 2-76 years***)
- **Weakness and atrophy of distal muscles** (feet >legs >hands), difficulty walking, sensory loss , impaired balance with **slow progression,** gradually increasing disability

- **Diagnosis** based on clinical course, family history/ inheritance pattern, nerve conduction studies (demyelinating vs. axonal), and genetic testing
- **Genetically heterogeneous: >100 causative genes!**

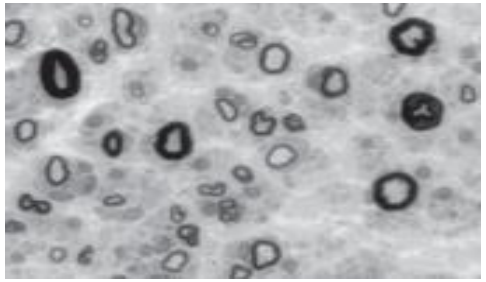
Genetic types and molecular mechanisms of CMT neuropathies

- **Diverse cellular functions**
- **Many disease mechanisms**

➢ **Schwann cell expressed genes:**
→ ***demyelinating CMT1, 4***



➢ **Neuronally expressed genes:**
→ ***axonal CMT 2***



Stavrou et al., 2020

Myelinating Schwann cell: CMT1, CMT4

Schwann cell cytoskeleton and linkage to extracellular matrix: *INF2, FGD4, PRX, FBLN5*

Transcription, mRNA processing: *EGR2, CTDP1*

Mitochondria *GDAP1, HK1*

Endosomal sorting and cell signalling *LITAF/SIMPLE, SH3TC2, MTMR2, MTMR13, SBF1, FIG4, DNM2, NDRG1*

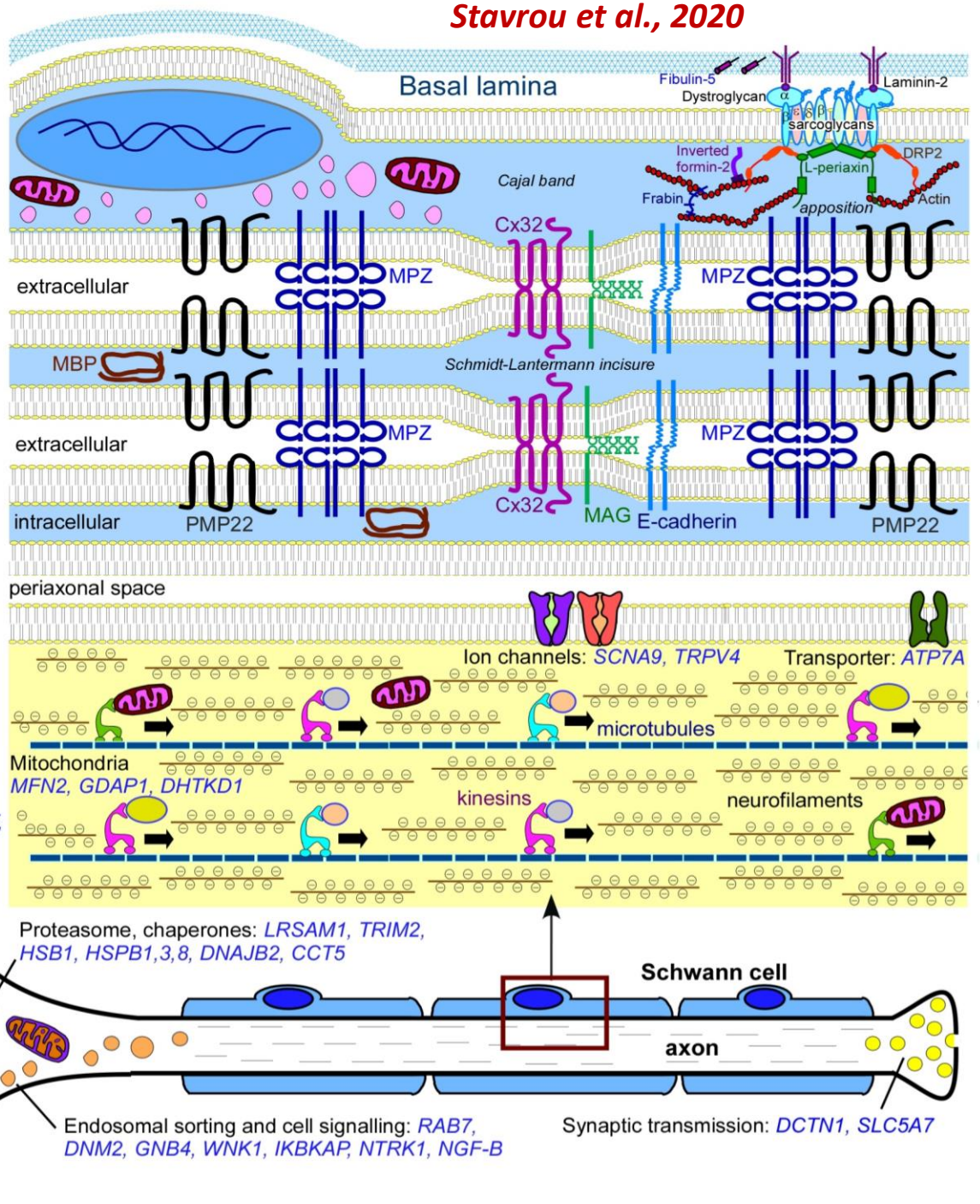
Compact myelin: *PMP22, MPZ*

Non-compact myelin: *GJB1*

Neuron and axon: CMT2

Axonal transport: *NEFL,NEFH, KIF5A, KIF1A, DYNC1H1, HSPB1, BICD2, MYH14*

Nuclear envelope, mRNA processing: *LMNA, GARS, AARS, YARS, KARS, MARS, HARS, TGF, HINT1, PRPS1, IGHMBP2, DNMT1, MED25, PLEKHG5*



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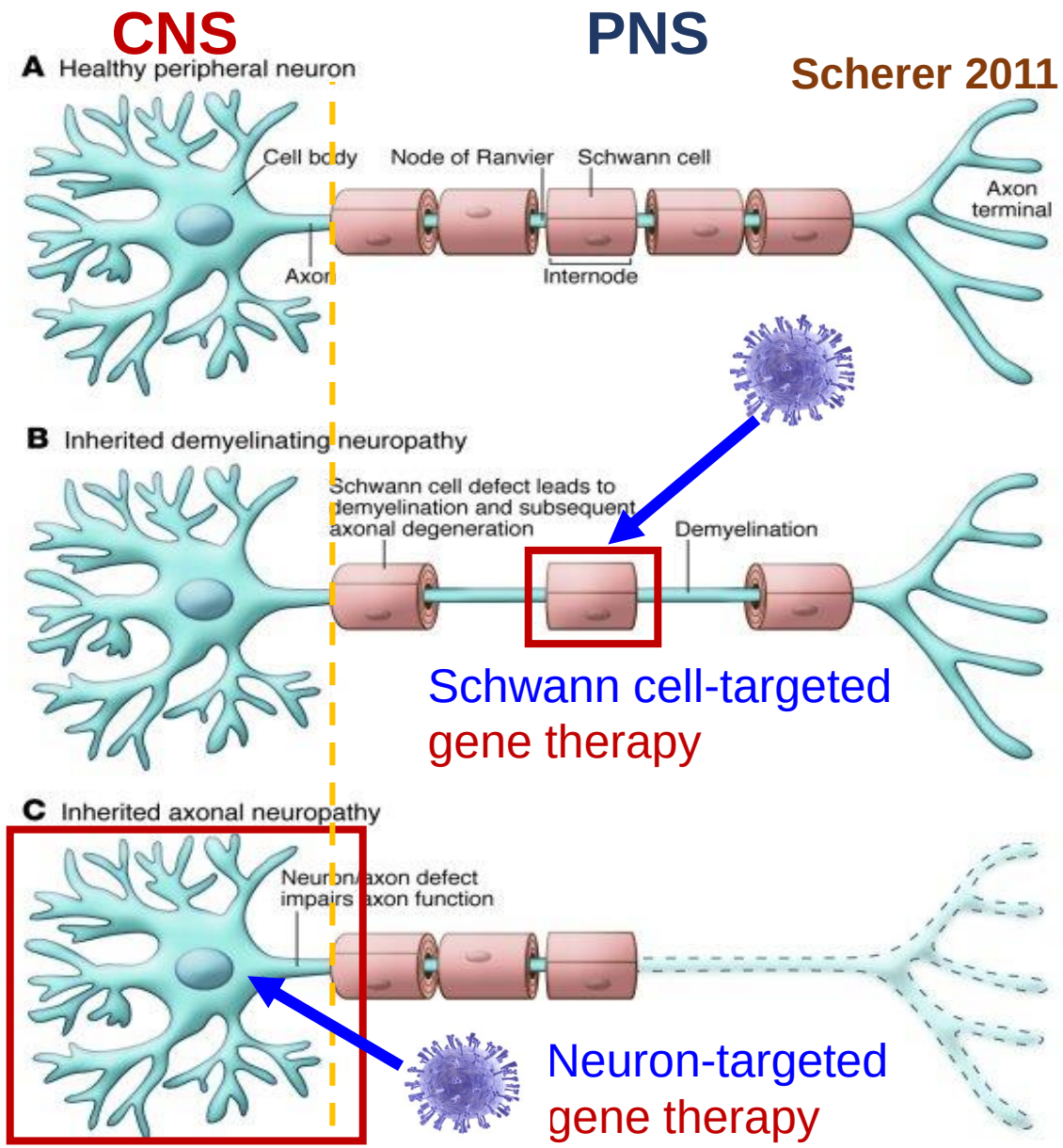
Planning gene therapies for CMT neuropathies



Gene therapies should:

- **address the disease mechanism:**
  - **Loss of function→ gene replacement**
  - **Gain of function→ gene silencing, allele-specific silencing, and/or editing**

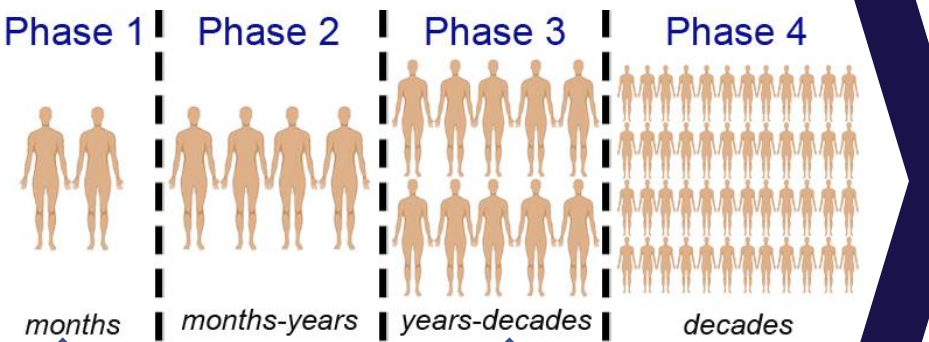
- **be delivered to the affected cell type:**

- Therapies for demyelinating CMT have to be **targeted to Schwann cells** *throughout the PNS*

- Therapies for axonal CMT neuropathies need to be **delivered to neurons** and their axons

Long route toward translation of CMT gene therapies

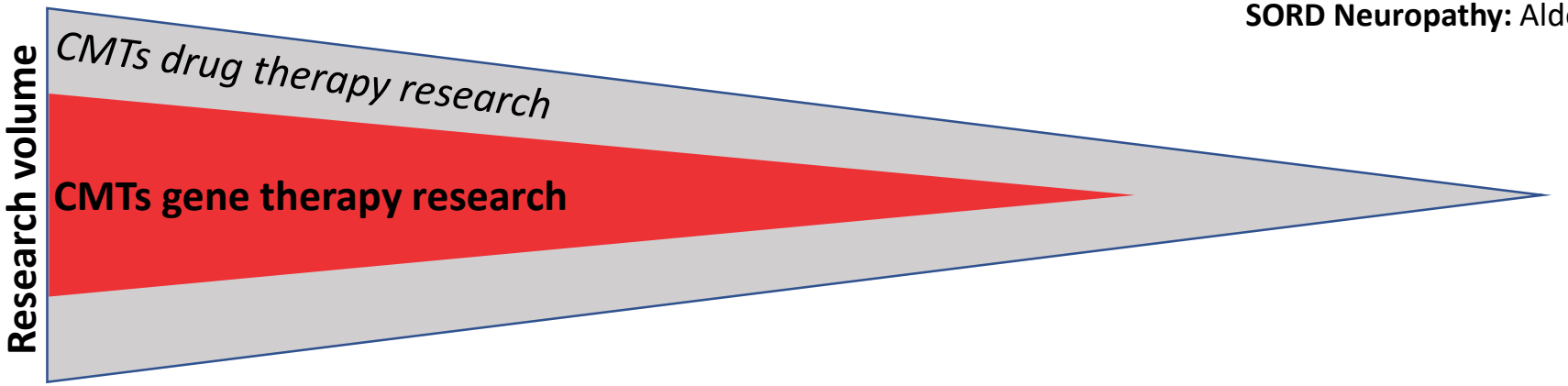
***In vitro* testing** ***In vivo* testing** **Testing in NHPs** **Clinical trial** **FDA approval**



CMT2S: AAV9-*IGHMBP2*
NCT05152823

CMT1A: PXT-3003 drug therapy
NCT05092841

SORD Neuropathy: Aldolase inhibitors

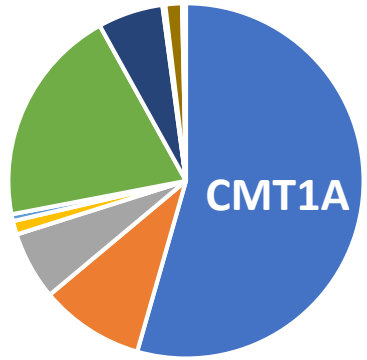


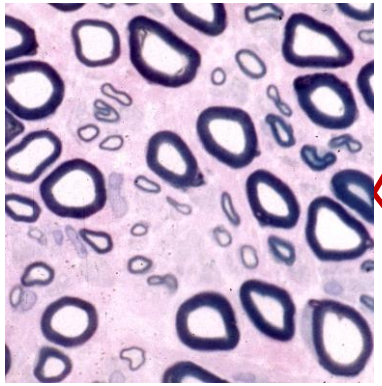
Currently, there is no EMA/FDA-approved gene/drug therapy for CMT neuropathies

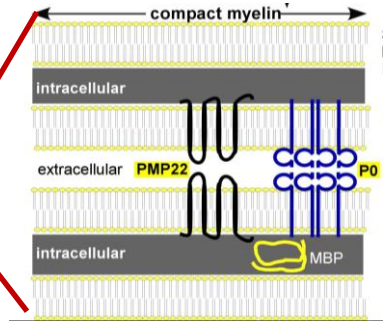
PMP22 duplication causes CMT1A

- **CMT1A** is the **most common** CMT type (~60 % of all CMT cases)
- Caused by peripheral myelin protein 22 **(*PMP22)* duplication**
- **Weakness and wasting** of distal muscles (feet >legs >hands), difficulty walking, sensory loss , impaired balance with **slow progression,** gradually increasing disability

CMT Subtypes by frequency

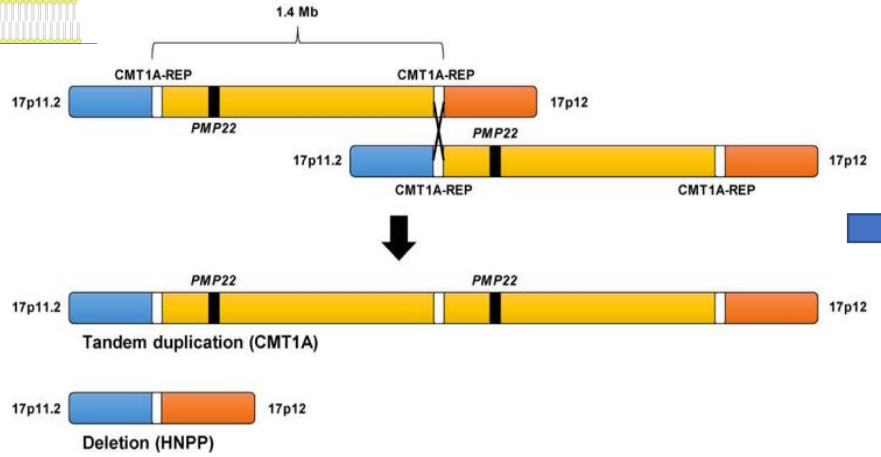






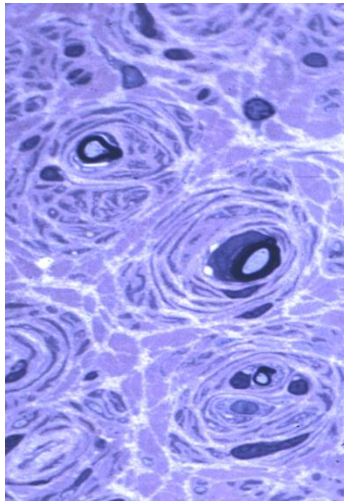
❑ **PMP22** regulates Schwann cell growth and differentiation, myelin formation and maintenance

Unequal crossing over during meiosis → 1.4 Mb duplication on chrom. 17 p11.2



➢ **PMP22 overproduction** leads to accumulation, decreased proteasomal activity, ER stress, myelin dysregulation and demyelination

→ **Increased gene dosage effect!**

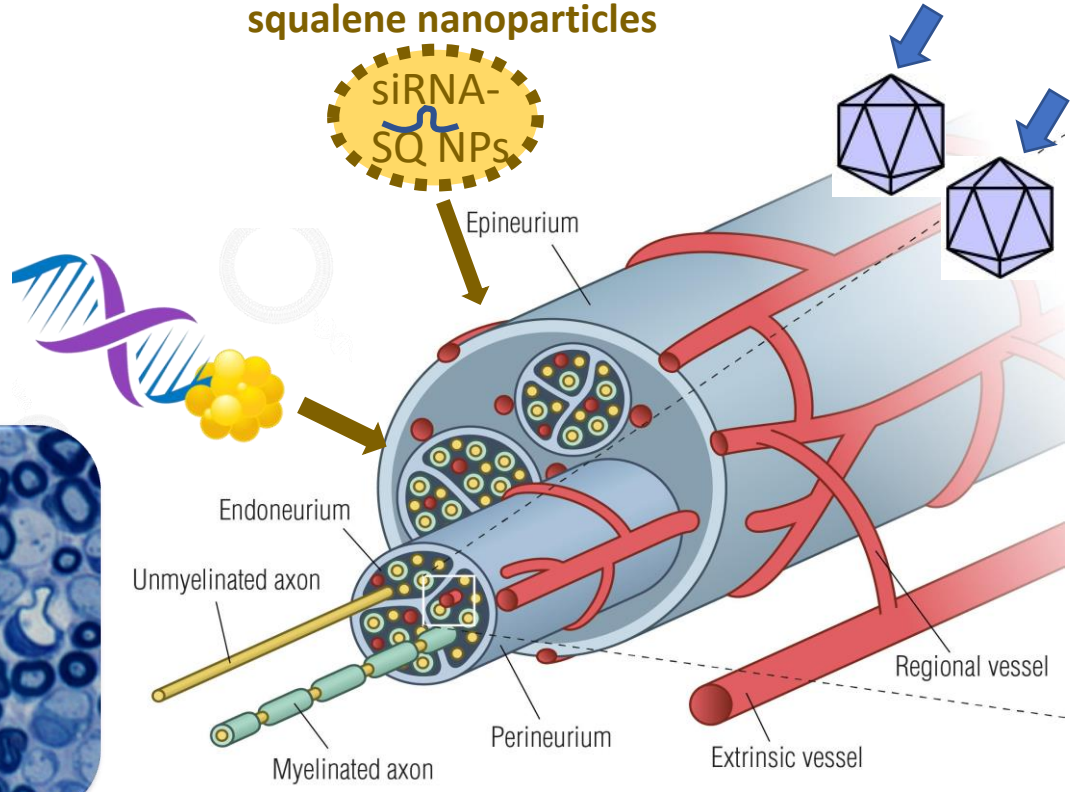


Gene therapies to reduce PMP22 levels

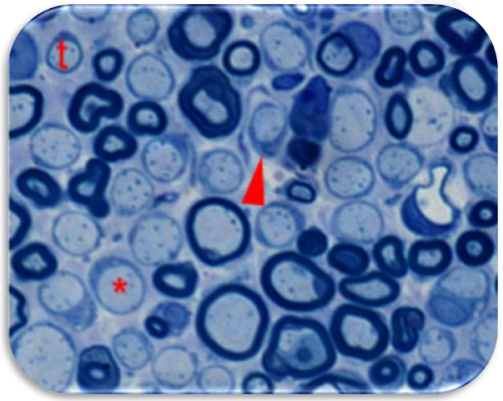
Non-viral methods

- Liposomes
- Nanoparticles
- ASOs

siRNA conjugated to **squalene nanoparticles**



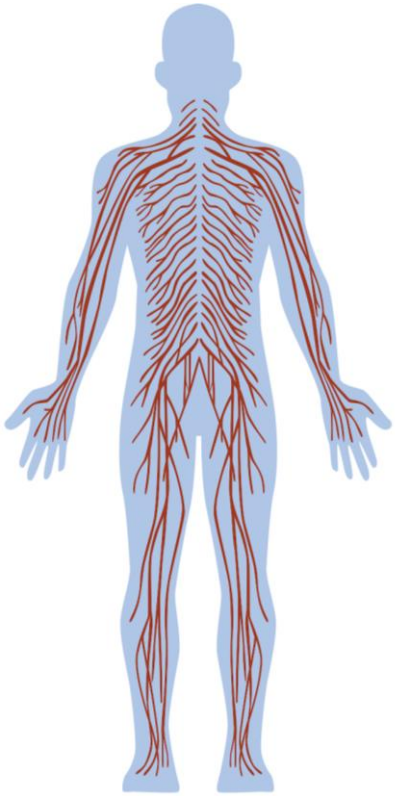
FALCON™ siRNA (Fatty Acid Ligand Conjugated OligoNucleotide)-DTx-Novartis



Viral vectors

AAV vectors

- delivery of silencing molecules: microRNAs, shRNAs, CRISPR-editing

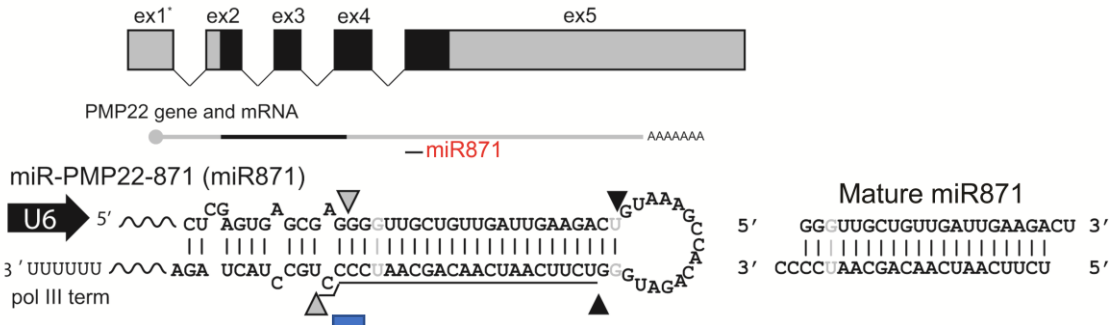


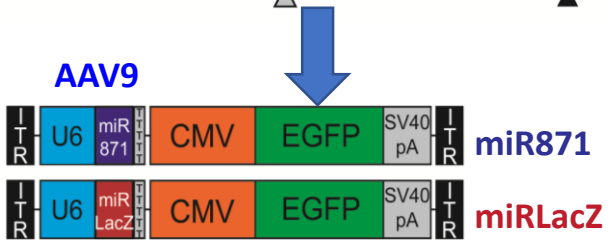
Delivery approaches for peripheral nerves

- **Subcutaneous** (ASOs, nanoparticles)
- **Intrathecal-preferable route for viral therapies**
- **Intravenous- high dose**
- **Intraneural** –restricted to injected nerve
- **Intramuscular**- for trophic factors

Development of gene silencing therapy for CMT1A

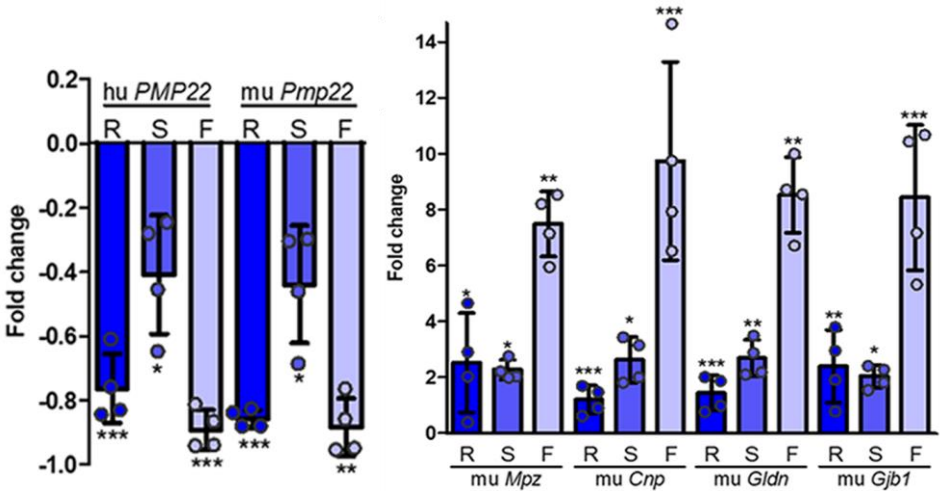
Stavrou et al., J Clin Inv 2022





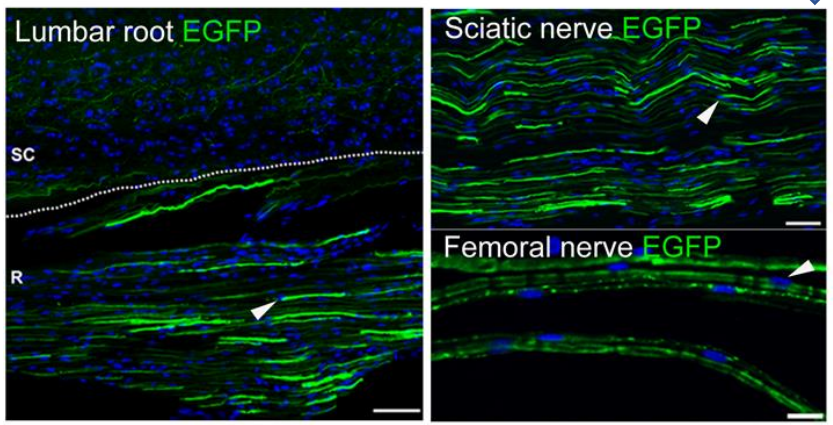
Intrathecal delivery: 5e11 vg/animal

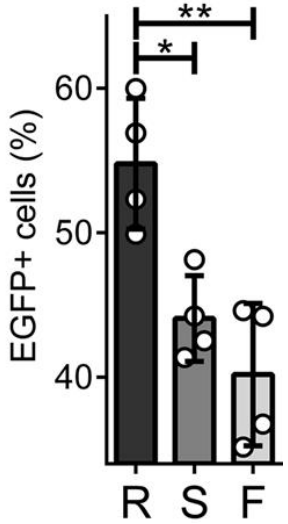
✓ **Selective reduction of PMP22 mRNA in the CMT1A model**



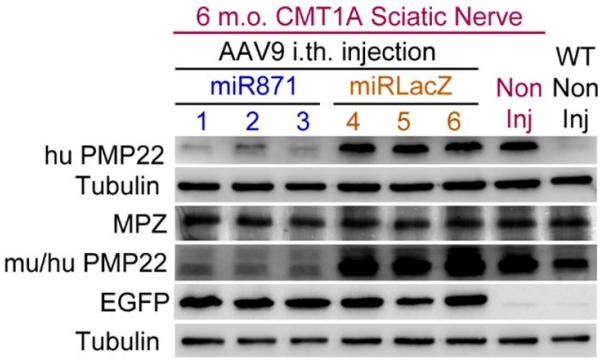
R: roots
S: sciatic nerve
F: femoral nerve

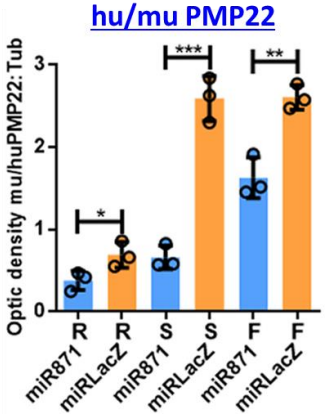
✓ **Strong targeting of peripheral nerves and Schwann cell expression**





✓ **Selective reduction of PMP22 protein levels**

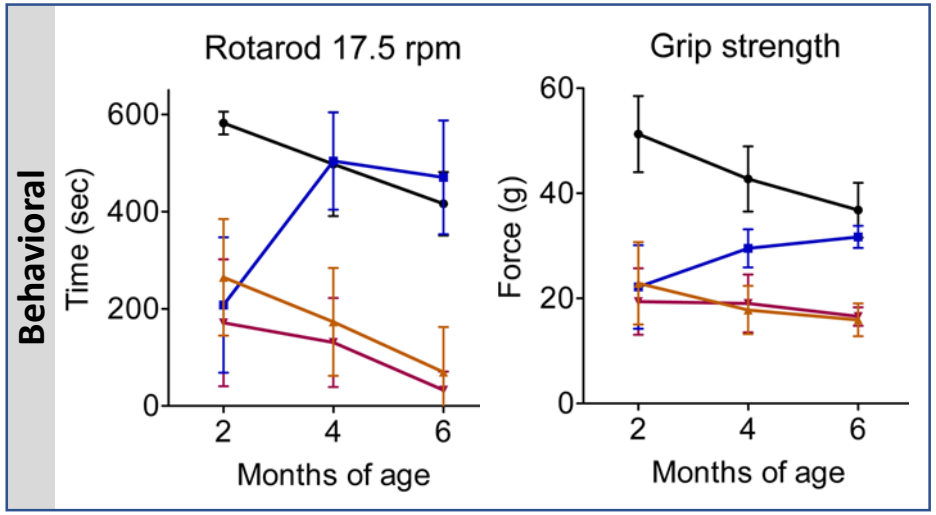




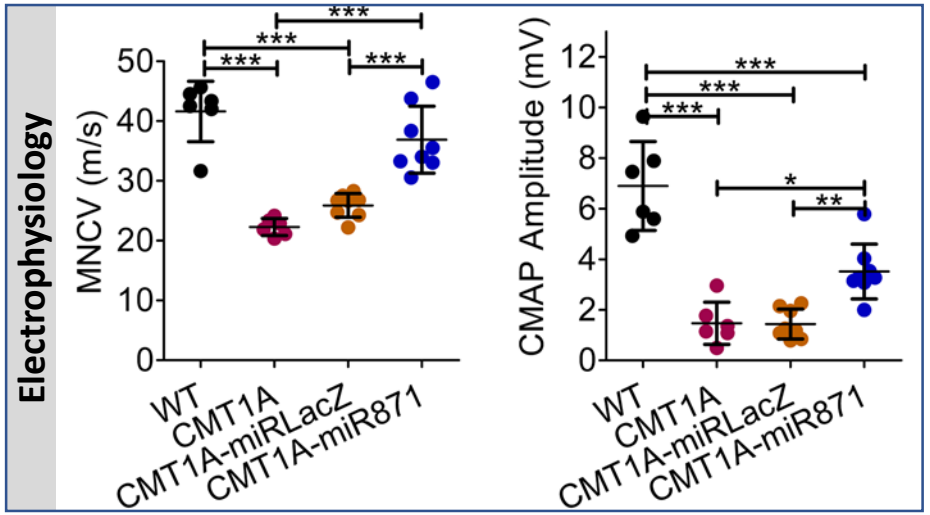
Development of gene silencing therapy for CMT1A

Stavrou et al., J Clin Inv 2022

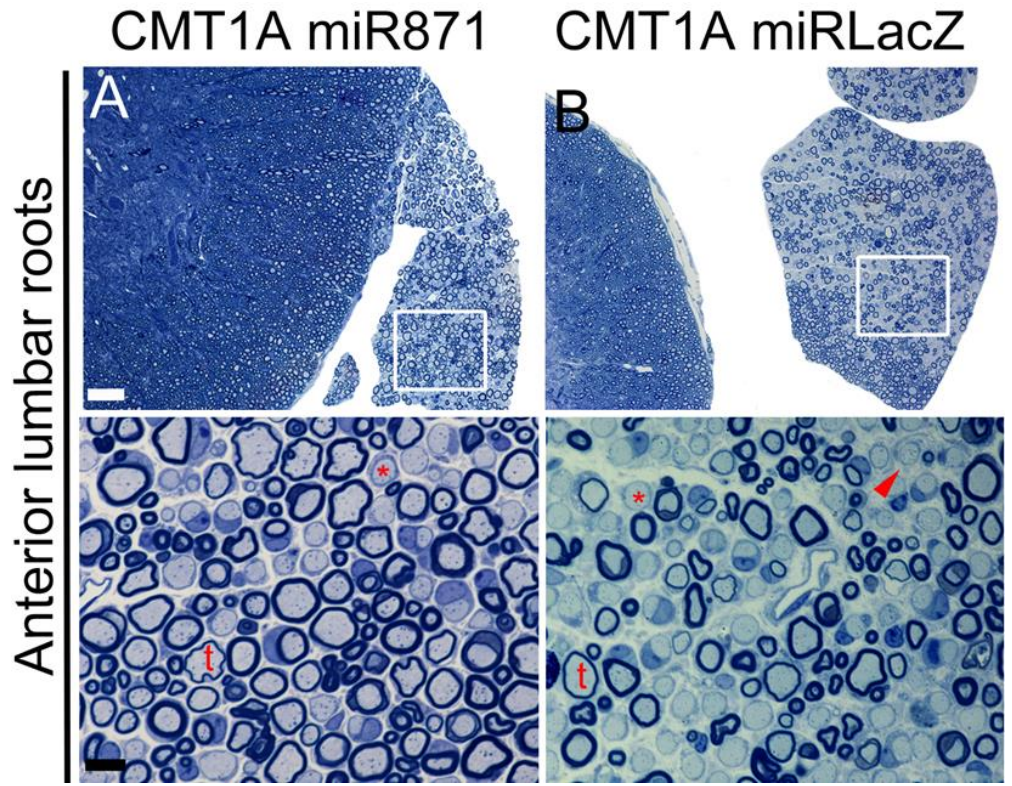
- ✓ **Improved motor performance of the CMT1A model**



- ✓ **Improved nerve conduction velocities and CMAP**



- ✓ **Improved myelination, in lumbar roots and peripheral nerves of CMT1A mice after AAV9-miR871 treatment**

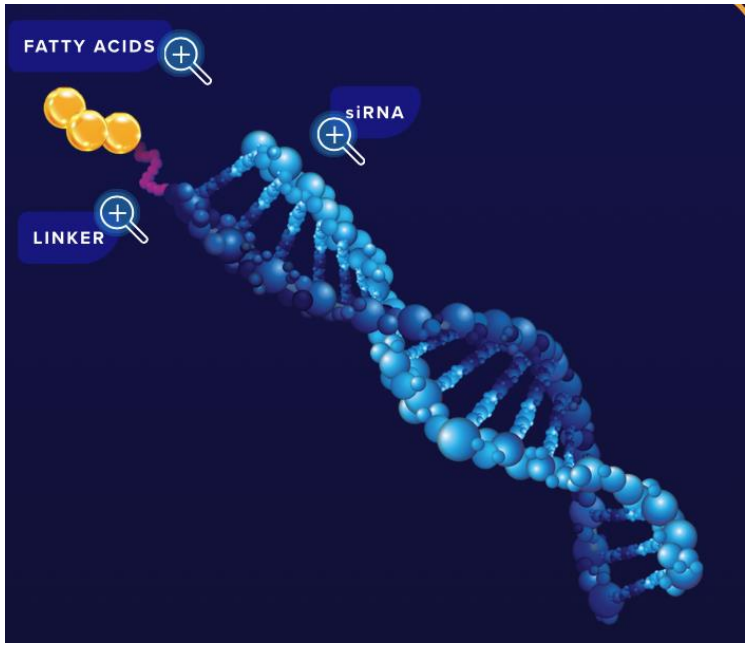


Fatty Acid Ligand Conjugated Oligonucleotides (siRNA) targeting PMP22

DTx Pharma (acquired by Novartis in summer 2023)

FALCON technology overview:

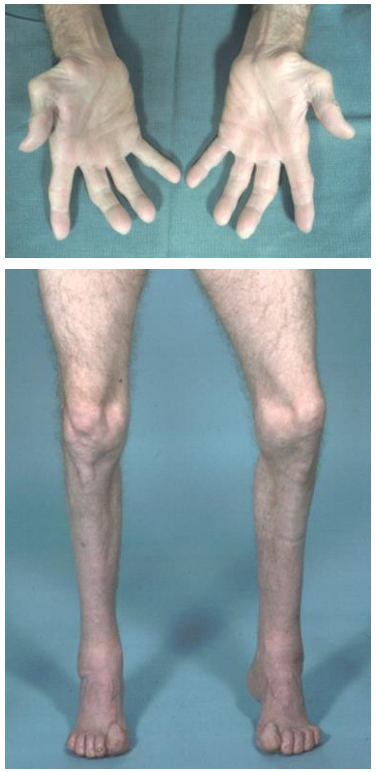
- **Conjugates of naturally occurring fatty acids to siRNA's→ improve cellular uptake and biodistribution through binding to fatty acid receptors**



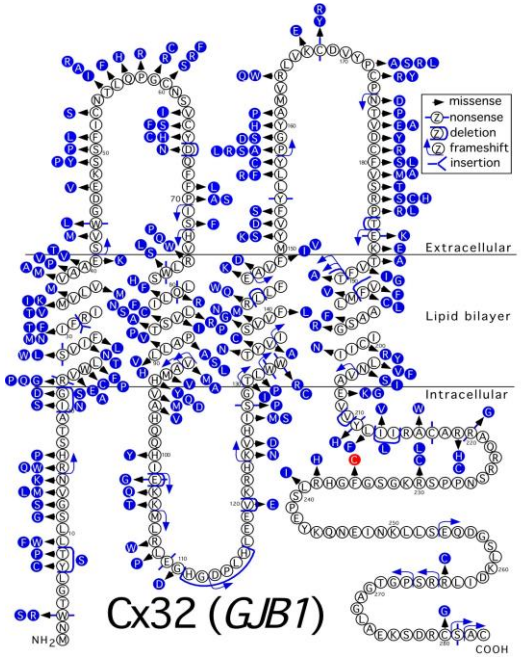
- ***In vivo* proof of concept using the C3 mouse model (overexpressing human PMP22) of CMT1A**
  - ✓ Remyelination of axons to normal levels
  - ✓ Improved muscle mass, grip strength, coordination and agility
  - ✓ "Reverse" multiple aspects of phenotype

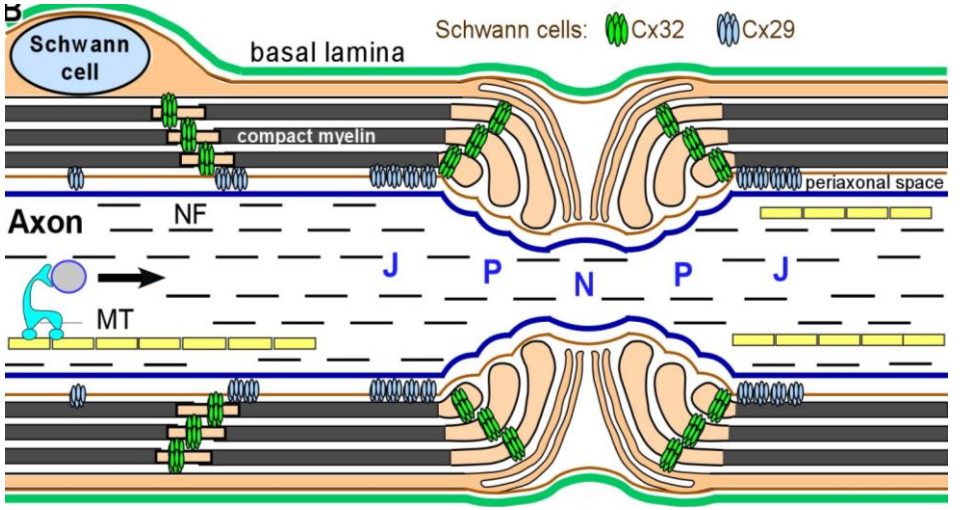
- Completed **GLP Tox Studies and IND filing**
- **Planning first-in-human dosing in 2024, with single and multi dose arms**
- **Outcomes:** Safety, Biopsy, Electrophysiology (to evaluate remyelination)

CMT1X phenotype and cause

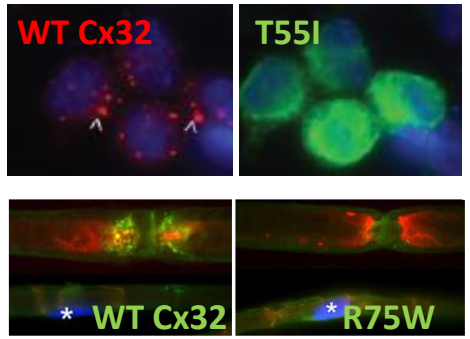


- **CMT1X: 2nd most common** form of CMT (15-20%) – estimated prevalence of **1:25,000**
- Caused by **mutations in the *GJB1* gene** (on chromosome **Xq13.1)** encoding the **gap junction protein Connexin-32 (Cx32)**
- **Men affected earlier and more severely**
- **Symptoms usually start between 5 and 20 years in men:** difficulty running, ankle sprains, muscle atrophy
- Slowly progressive





- **Cx32 forms gap junction channels** through the myelin layers of myelinating Schwann cells in peripheral nerves
- **Communication pathway** transversing the myelin sheath **preserving homeostasis, axon support**



➢ In **CMT1X loss of Cx32 gap junction function leads to progressive degeneration of myelin and axon**

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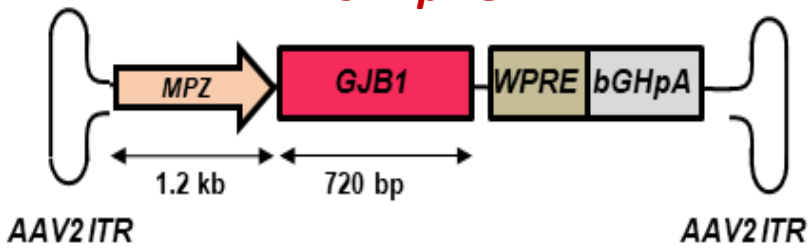
AAV-mediated *GJB1* gene replacement

Kagiava et al., Sci Rep 2021;
Kagiava et al. Gene Therapy 2021;
Kagiava et al., Mol Ther Meth Clin Transl 2023

Therapeutic vector:

AAV9-*Mpz.GJB1*



Myelin-specific myelin protein zero (Mpz) promoter

→ Lumbar intrathecal delivery in different models of CMT1X $2x10^{11}$ vg

- **Adequate biodistribution to PNS tissues**
- **High percentage of Schwann cell-specific gene expression**
- **Pre-onset (2 month) and post-onset (6 month-old) treatment trial in CMT1X models provides therapeutic benefit**

➢ **Cx32 deficient (*Gjb1*-null)**

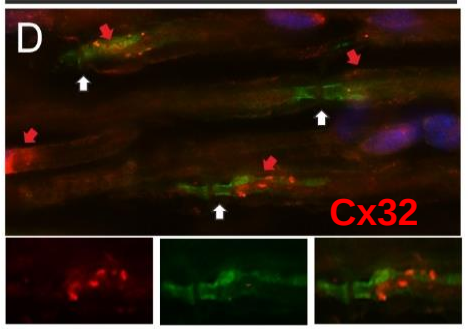
➢ **CMT1X transgenic (R75W and N175D):**

✓ **Functional improvement** (muscle strength, nerve conduction velocities)

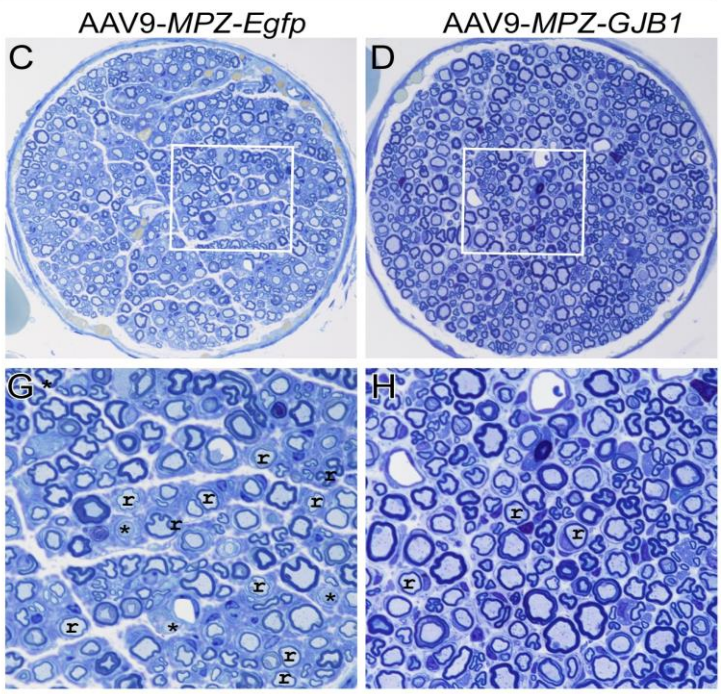
✓ **Improved myelination** (reduced demyelinated and thinly myelinated fibers)

✓ **Reduced inflammation** (number of macrophages)

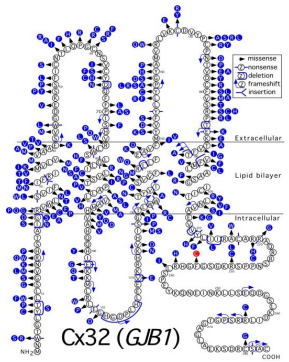
Gjb1-null/AAV9-*Mpz.GJB1*



N175D KO



Route toward translation of CMT1X gene therapy



➢ **Disease cause and mechanisms**

In vivo testing in disease models

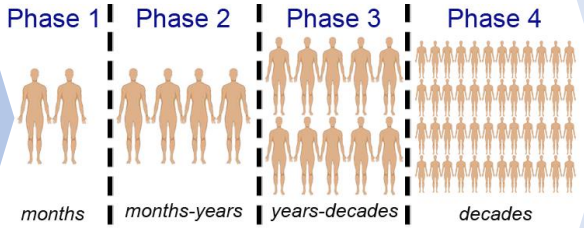
- **POC studies completed → therapeutic benefit of IT AAV9 and AAVrh10 in different CMT1X models**

- **Dose-escalation and toxicity study completed successfully in the CMT1X model**
- **Further testing of leading capsids underway**

Testing in NHPs

- **Non-human primate studies to assess scale-up potential and safety in collaboration with industry**
- **Completed 12/2023, final results Q2 2024**

Clinical trial



- **Natural history studies**
- **Clinical trial readiness**
- **Biomarker validation (MRI, etc)**

FDA approval

Gene therapy for SMA with respiratory distress type 1 (SMARD1)/ CMT2S

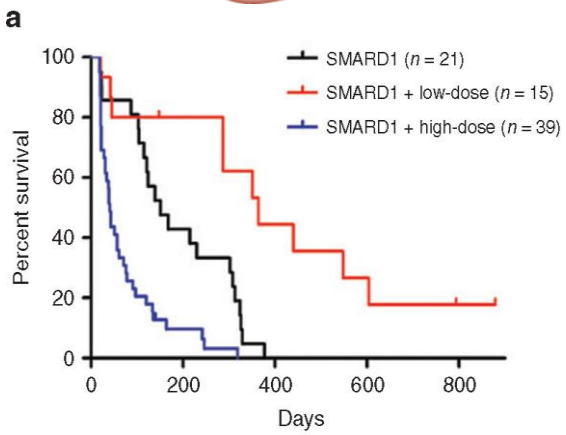
- **Recessive loss of function *IGHMBP2* mutations** → lower motor neuron loss → limb muscle atrophy and respiratory complications in infancy (**SMARD1**)
- **Partial loss of function** → milder phenotype with later onset: **CMT2S**
- **Dose-dependent gene replacement in the SMARD1 mouse model (*nmd*)**

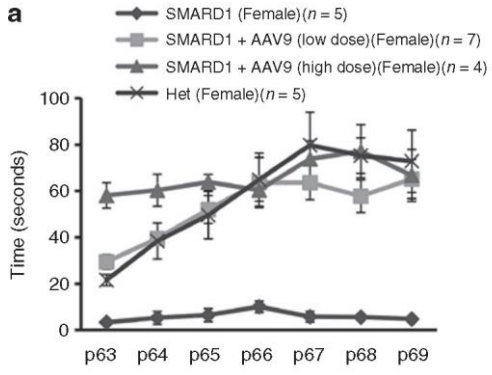
- ***Clinical Trial*** NCT05152823 ***by Alkyone Therapeutics in children 2 mo-14 yr***

Shababi et al., 2016, Mol Ther

1.25-2.5e11 vg/animal

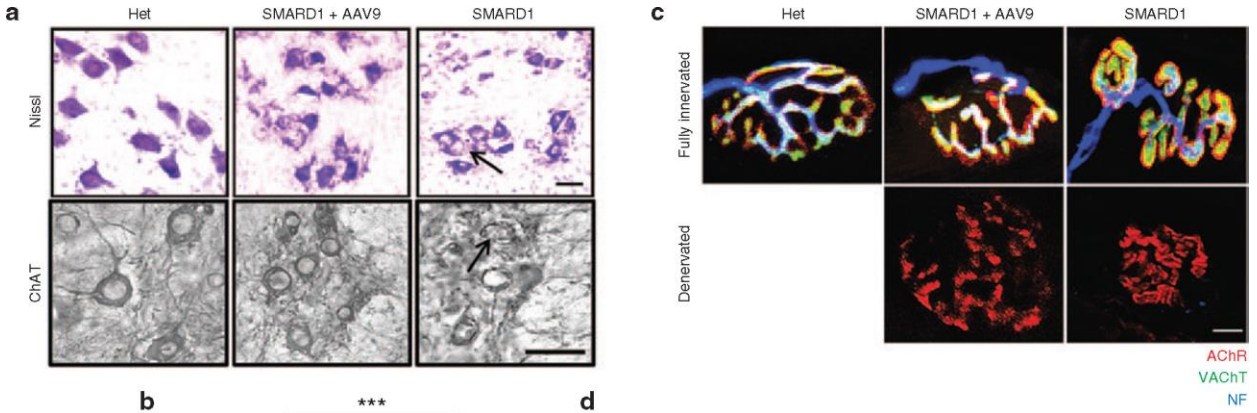
AAV9-CBA-IGHMBP2
post natal day 1-4

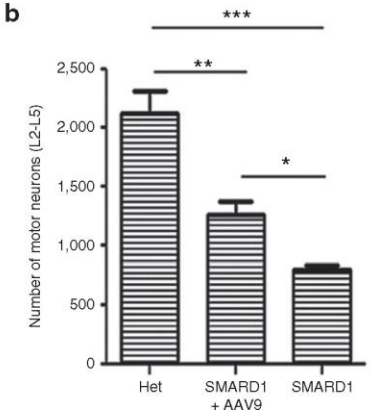


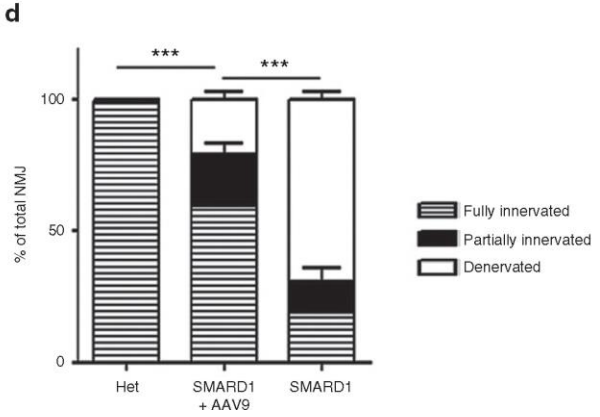


- **AAV9-IGHMBP2 *low but not high dose* increased survival and improved motor function**

- **AAV9-IGHMBP2 rescued loss of motor neurons and NMJ innervation**







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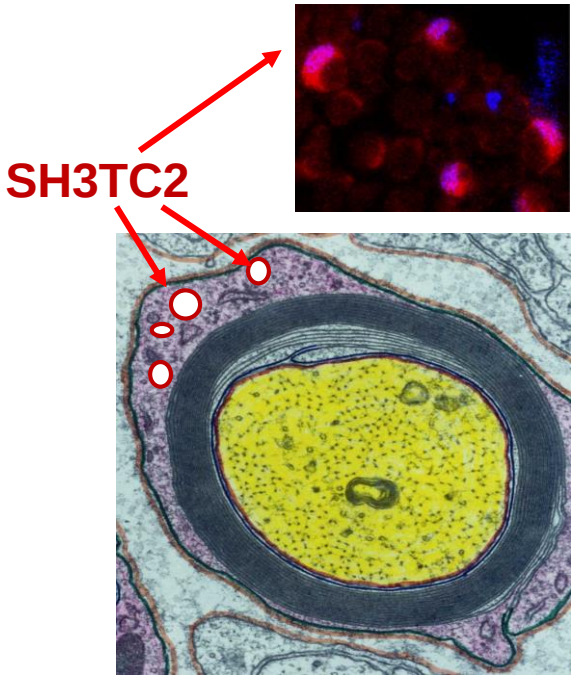
Gene therapy for CMT4C

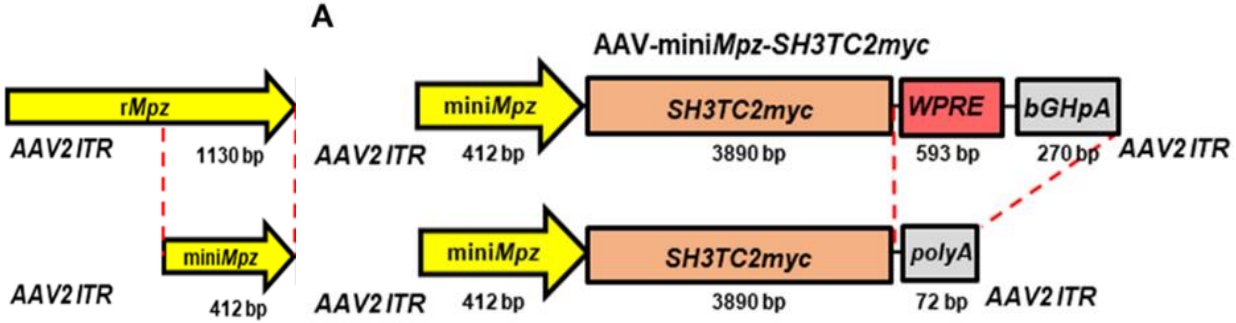
- **CMT4C: Severe, early onset demyelinating** CMT neuropathy with faster **progression** compared to CMT1 forms
- Caused by mutations in the ***SH3TC2*** **gene -most frequent CMT4 type: 3-4% of all non-CMT1A demyelinating CMT neuropathies**
- Autosomal recessive with **loss of function** mechanism

- **SH3TC2** protein is an endocytic recycling compartment protein specifically expressed by myelinating Schwann cells –interacts with Rab11 → **CMT4C mutations disrupt function**
- Early onset demyelination in ***Sh3tc2*** $^{-/-}$ mice *(Arnaud et al., 2009)*



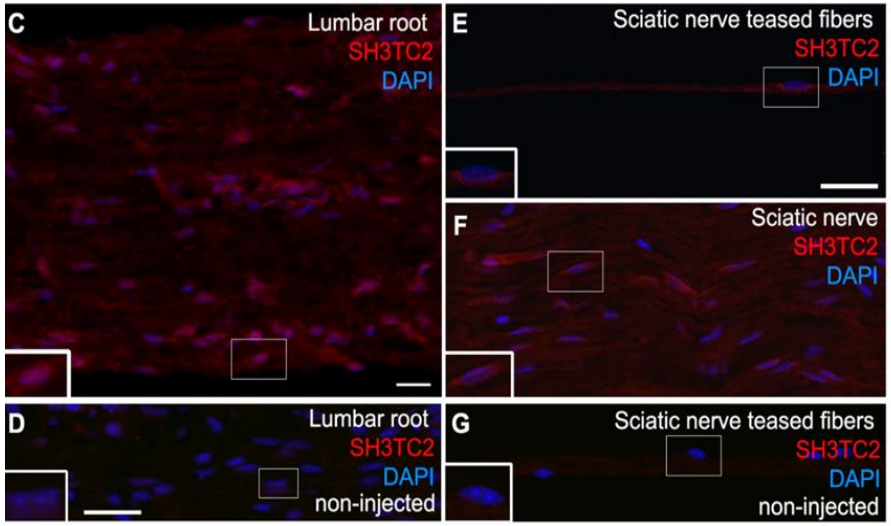
➢ Therapeutic vector generation for SH3TC2 gene replacement

Georgiou et al., Mol Ther 2023



Sh3tc2-/- mice

➢ **Lumbar intrathecal injection** 20 µl (2E+11 vg) in *Sh3tc2*-/- mice



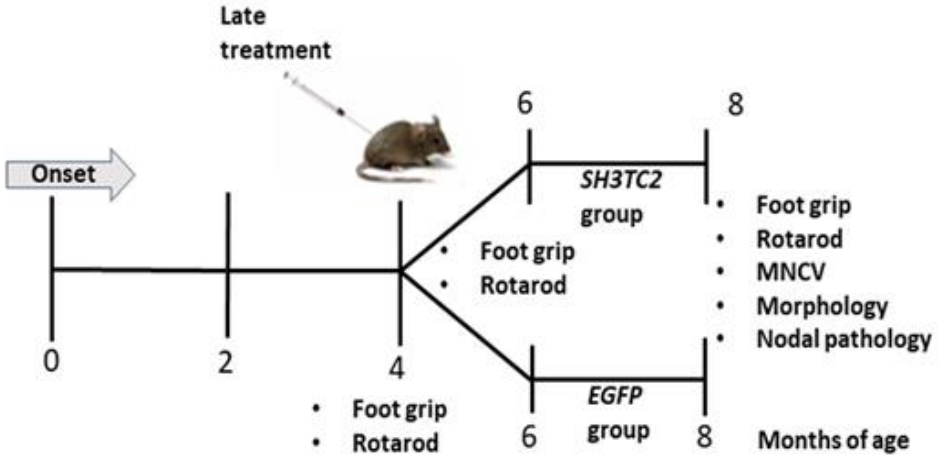
➢ *Sufficient* ***vector biodistribution to the peripheral nerves***

➢ *High* ***SH3TC2 expression rates in Schwann cells***

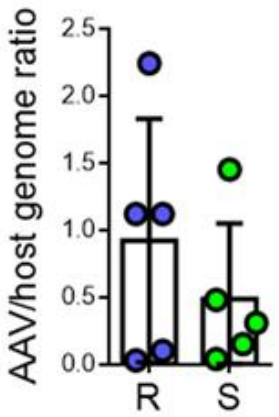
Functional improvement in treated *Sh3tc2*-/- mice

Georgiou et al., Mol Ther 2023

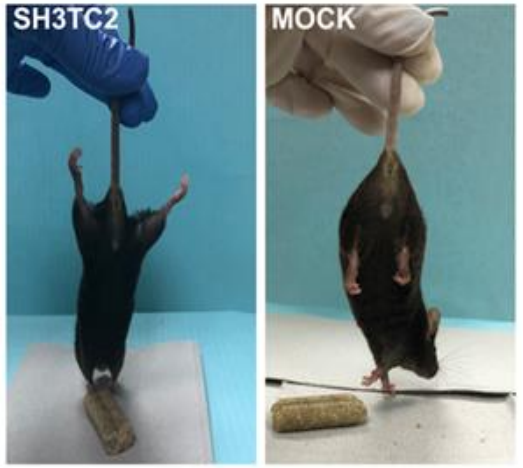
Study design

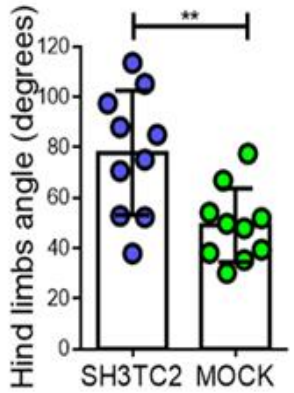


Biodistribution

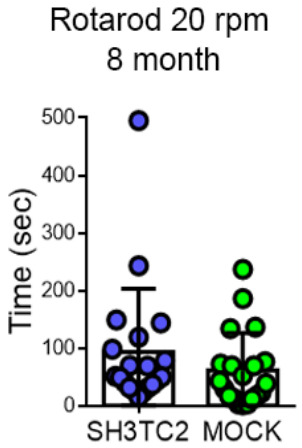


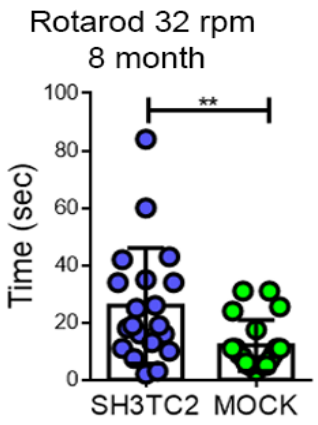
Behavioural analysis

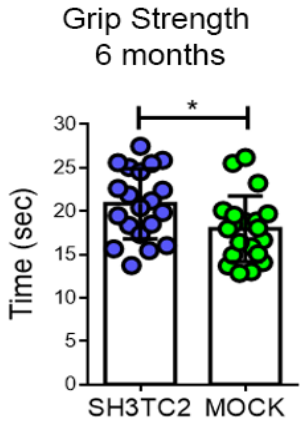


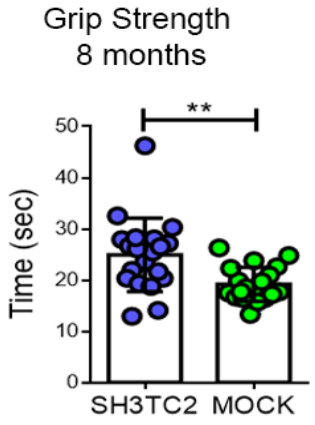


Behavioural analysis

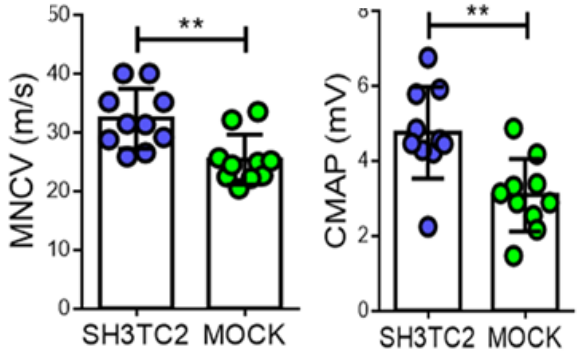






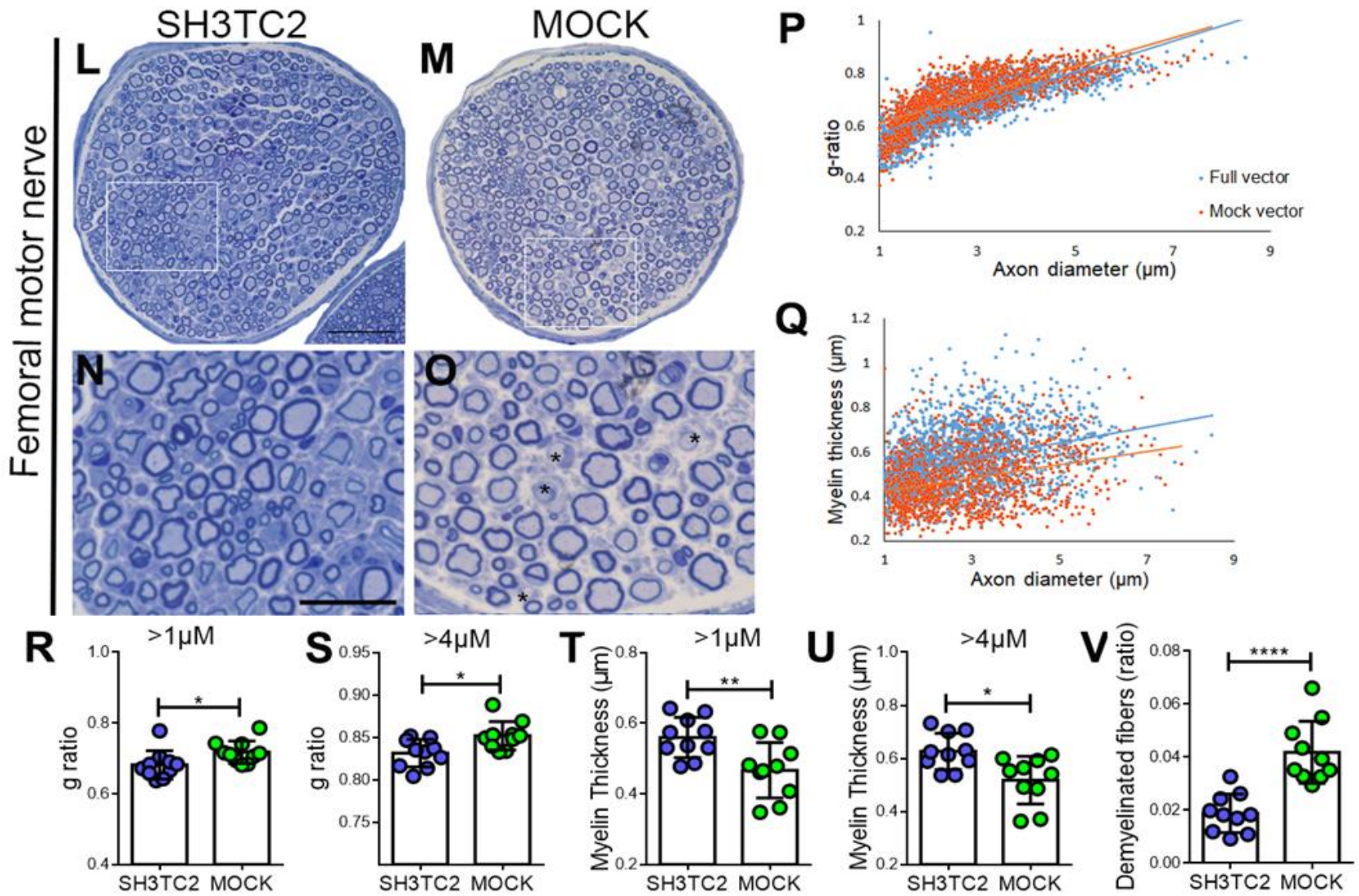


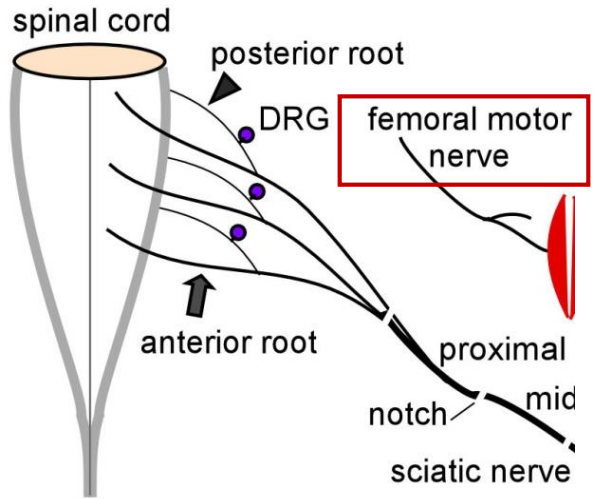
Electrophysiology



Improved myelination in treated *Sh3tc2-/-* mice

Georgiou et al., Mol Ther 2023





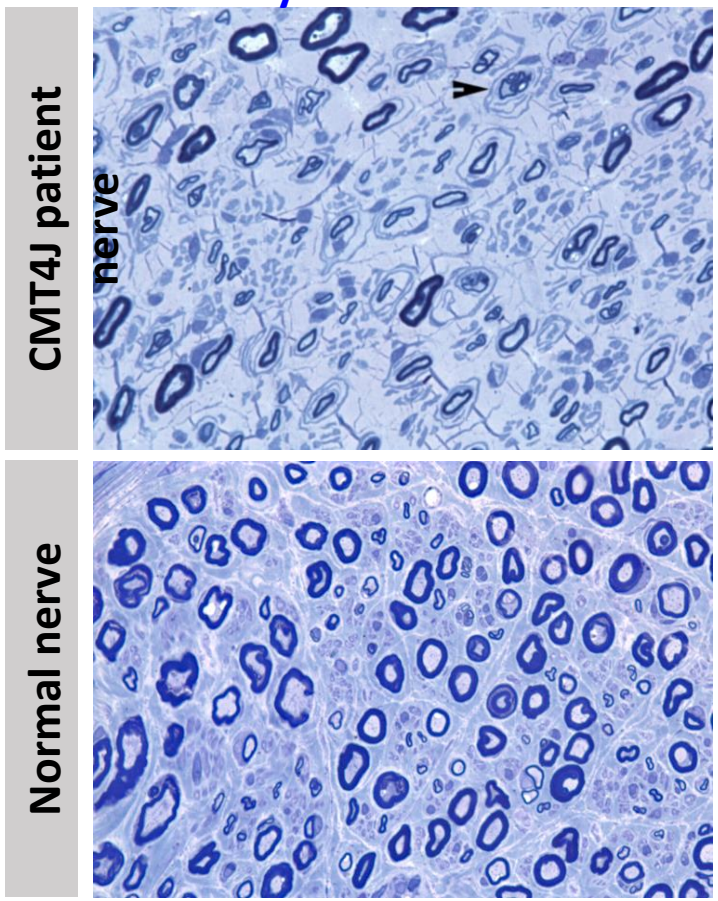
- **Similar results in lumbar motor roots and in sciatic nerves**
- **Similar results with early treatment**

Other CMT4: CMT4J

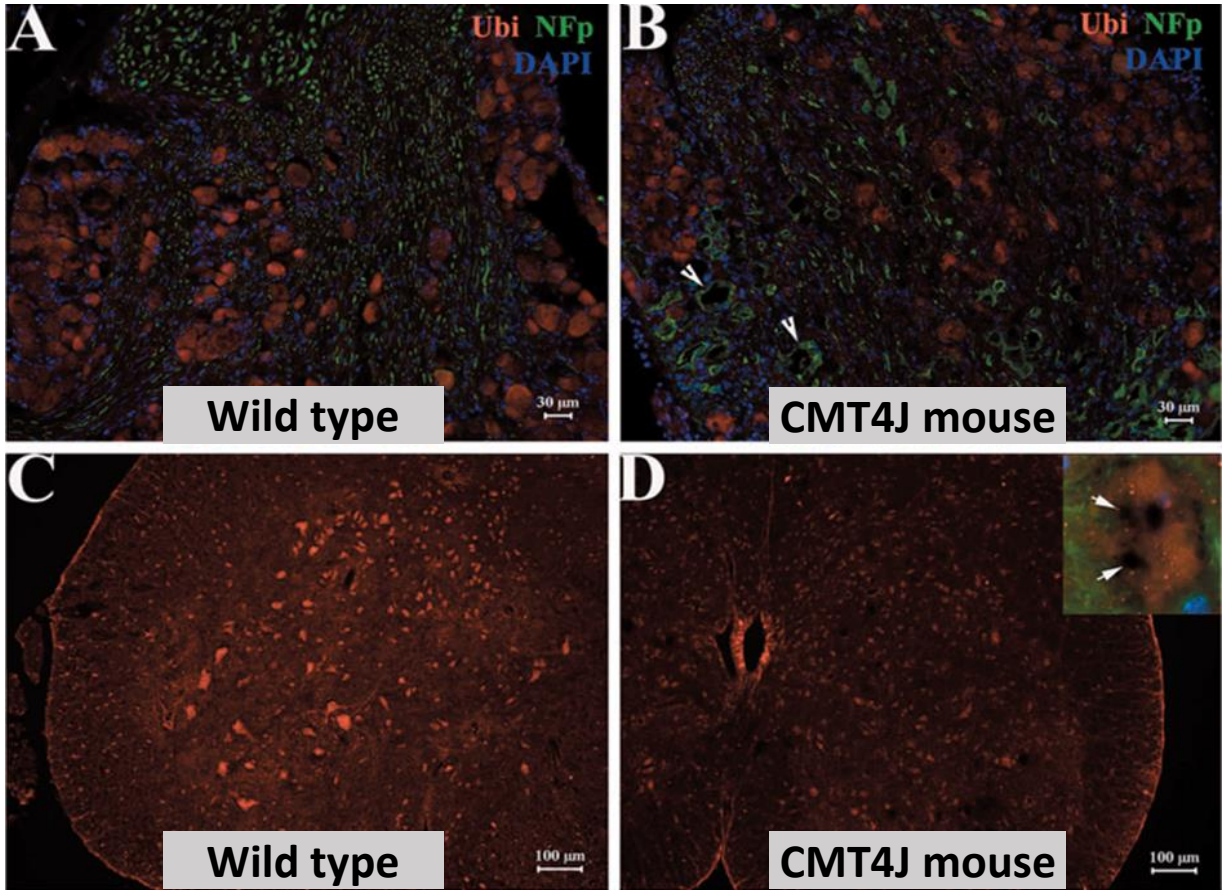
- CMT4J is the **0.3% of all CMT cases**
- Caused by **loss of function mutations in *FIG4*** gene
- FIG4 is 5-phosphate serves in **endosome/lysosome pathway**

Zhang et al, 2008, Brain

Demyelination in CMT4J



Loss of large motor and sensory neurons in CMT4J



CMT4J gene therapy: AAV9-CBA/CMV-*FIG4*

CURE CMT4J
ADVANCING TREATMENTS FOR RARE DISEASES

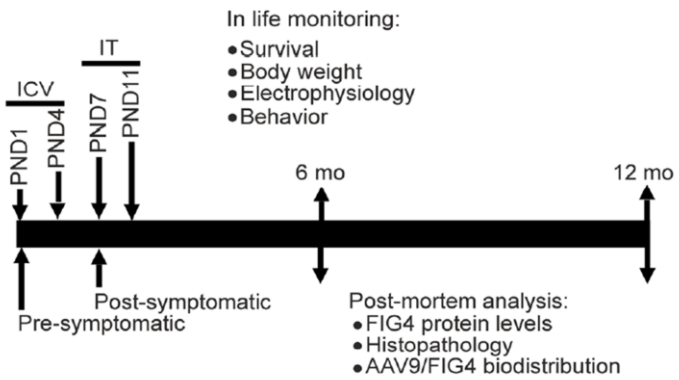
Presa et al., 2021, J Clin Invest

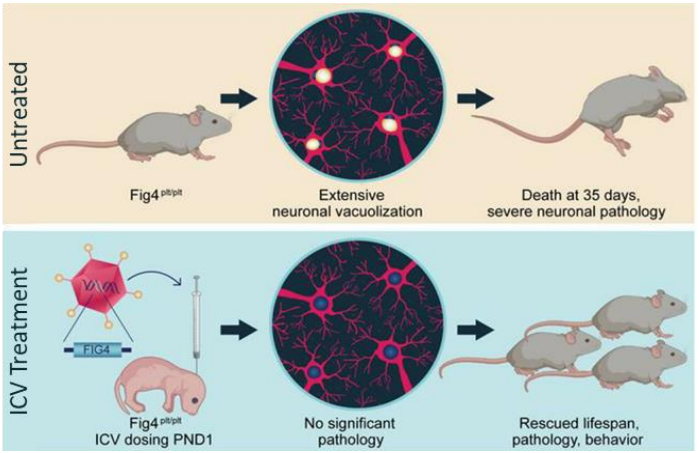
Fig4–pale tremor (*plt*)

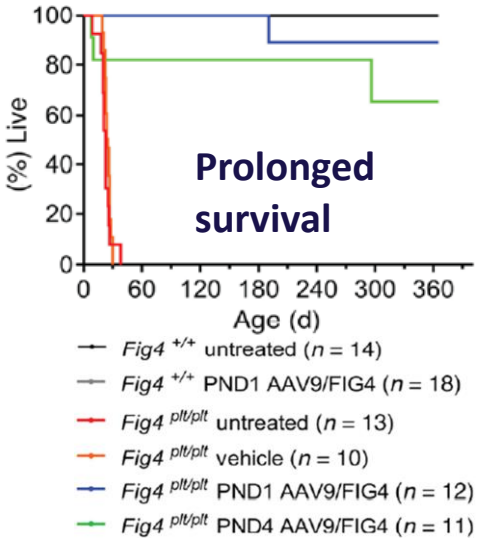
Transposon insertion
in intron 18 of mu *Fig4*

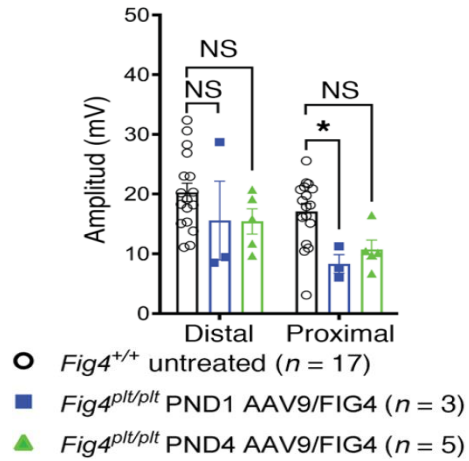
AAV9-CMV/*CBA*-*FIG4* treatment:
postnatal day 1 or 4

ICV: 5.4e11 vg/animal





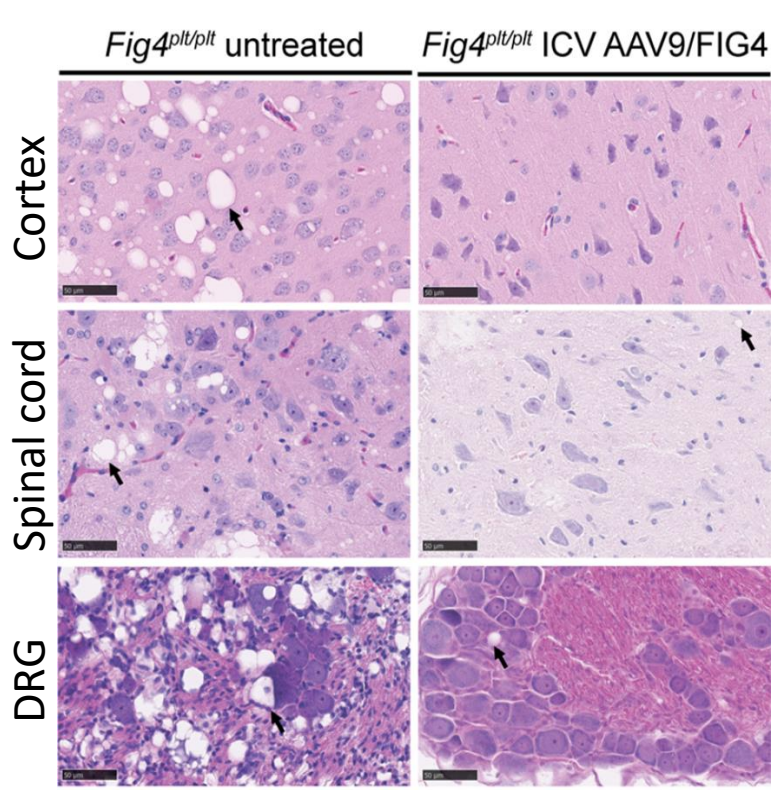




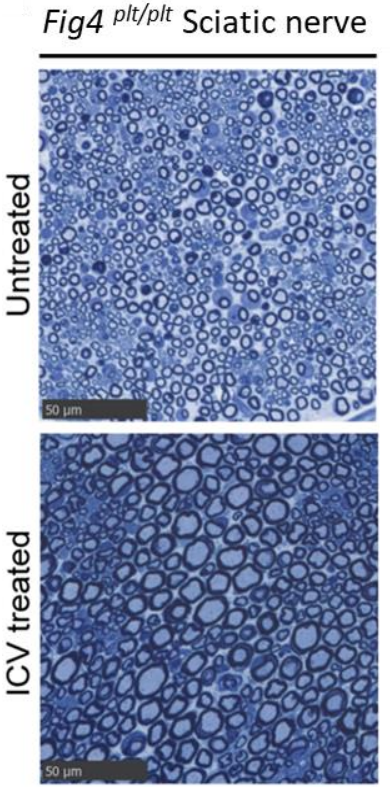
CMT4J gene therapy:
→ clinical trial, advocated by CureCMT4J, now part of Bespoke Gene Therapies Consortium

- **P1 or P4 Tx→ survival > 1 year, normal motor**
- **P7 or P11 Tx→ prolonged survival and function *but incomplete***

Rescue of motor neurons



Improved myelination



Opportunities and challenges in gene therapy for neuropathies

Challenges in clinical translation

- The **earlier the intervention** the more beneficial the treatment will be, but have **to get the dose right!**
- CMT neuropathies **are non-life threatening diseases**, need to **ensure that "potential treatments" are safe**
- **Need for sensitive clinical outcome measures** and **treatment responsive biomarkers**
- Need **natural history data for ultra-rare forms, biomarkers** for all CMT types!
- **Industry limitations**, financing clinical translation
- **Regulatory requirement**s and limitations

Opportunities

- ✓ **Strong advocacy Groups, Patient Associations**
- ✓ **Progress in Natural History Studies, biomarker discovery**
- ✓ **Collaborative Network** between scientists, clinicians, patients, industry
- ✓ **Increasing experience in clinical gene therapies and safety aspects**

THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

- **Steven S. Scherer, MD, PhD,** Univ. of Pennsylvania, PA
- **Scott Harper, PhD,** Nationwide Children's Columbus, OH
- **Mary Reilly, MD and Alex Rossor, MD, Henrik Zetterberg, PhD**, UCL, London
- **Rita Horvath, MD,** Univ. of Cambridge, UK
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- **Assumpció Bosch, PhD,** Universitat Autonoma of Barcelona, Spain
- **Steven Gray, PhD**, UTSW, Dallas, TX

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Thank you !

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